Review Article

BIOLOGICAL AND PHYTOCHEMICAL INVESTIGATIONS RECENTLY CARRIED OUT ON THREE *VIBURNUM LINN*. SPECIES – AN OVERVIEW

*,1Prabhu K, 2Karar P K, 3Hemalatha S, 1Ponnudurai K
*,1Nandini Nagar Mahavidyalaya College of Pharmacy, Nawabganj, Gonda, Uttar Pradesh, India - 271303.
2Doaba College of Pharmacy, Kharar, Mohali, Punjab, India - 140 103.
3IT-Banaras Hindu University, Varanasi, Uttar Pradesh, India - 221 005.

Abstract
In ancient Indian medicin literature right from 19th century A.D, several species under the genus viburnum Linn. Belonging to adoxaceae family have been recognized for their medicinal properties from very early times of this century, the magnitude of scientific studies, especially pharmacognostical, phytochemical and biological, have been in record. However, the numbers of viburnum species subjected to scientific investigations are very limited (about 200 species in the world and 17 of them in India). Hence this article list out some phytochemical and biological studies so far undertaken on the leaves,stems and the roots of three *Viburnum* species(Namely: *Viburnum punctatum* (VP), *Viburnum coriaceum* (VC) and *Viburnum erubescens* (VE)) to the date and the current status of the plant in the world, with an objective that this paper may create awareness among pharmcognosist to progress pharmacogonostical and phytochemical based pharmacological studies in future.

Keywords: *Viburnum, punctatum, coriaceum, erubescens*, Adoxaceae.

Introduction

India has a rich heritage of using medicinal plants and hosting several thousands of medicinally valuable plants belonging to hundreds of families. One cannot assure that all of these plants possess a long recorded history, although they have been reported to contain medicinally valuable phyto-pharmaceuticals. For many of them, an authentic protocol derived from multidisciplinary approach is very scant. In particular, the plants, which are growing at elevated altitude ascending more than 2000 ft and forest dominated hilly areas, are not exposed to plant vendors, botanists, plant collectors and pharmacognosists due to inaccessibility and climatic conditions of the locations. The genus *Viburnum* Linn. is a typical example of such a kind, which is dwelling at a high altitude, belonging to the family Caprifoliaceae.

Author for Correspondence:
Prabhu K,
Nandini Nagar Mahavidyalaya College of Pharmacy,
Nawabganj, Gonda, Uttar Pradesh,
India - 271303.
Email: prabhu.cognosy@gmail.com

The genus *Viburnum* Linn. includes about 17 species in India and about 200 species distributed throughout the world[1,2]. *Viburnum* Linn. Species have been reported to contain sesquiterpenoids, triterpenoids[3] and sterols; phenolic compounds and their glycosides such as tannins, flavonoids and anthocyanins and irridoid glycosides in their stem, root and leaves, and investigated to posses uterine sedative, diuretic, cardiovascular stimulant, antimicrobial, anti-inflammatory, anti-nociceptive, antispasmodic, anti-asthmatic and astringent activities[4,5]. In the late 1960s and early 1980s, scientific studies on the genus *Viburnum* Linn. were voluminous[6,7,8]. However, the number of species subjected for studies and areas of investigations were narrow. After a couple of decades, a few *Viburnum species* reemerged to undergo some extensive phytochemical and pharmacological investigations. The typical examples are: irridoid aldehyde and their glycosides in *Viburnum luzonicum* and their cytotoxic effect[9]; vibsane type diterpene from *Viburnum awabuki*[10]; irdoid glycosides from *Viburnum tinos;* antinociceptive and anti-inflammatory activities of *Viburnum lanata*[11] and *Viburnum opulus*[12]; iridoid glucoside from *Viburnum rhytidophyllum*[13] .As a

whole, an overall utilization to scientific study of the genus as depicted(Figure 1) may be stated scant.

In addition to the above, a questionnaire and verbal enquiries to the local dwellers, tribals and herbalists of Nilgiri hills and Coimbatore hills (Top Slip) Tamilnadu, about some *Viburnum* species, were conducted; for it has been noted that the leaves, stem bark and root barks of mature plants had been reliably in usage to uterus and GIT related ailments, and still in practice, as an ideal healing aid as well as one of the best home remedies. As far as the species of priority is concerned *Viburnum coriaceum* (VC) (Leaves and Stem bark), *Viburnum erubescens* (VE) (Stem and roots) and *Viburnum punctatum* (VP) (stem and roots) are significant.Hence,the current article centres on the recent scientific studies conducted on the later during the period 2009-2011.

General botanical features of the genus – *Viburnum*

A evergreen tree or extremely variable shrubs, or medium sized evergreen trees; leaves elliptic-obovate Coriaceous, punctate base; acute, to cuneate,apex shortly acuminate lateral nerves 6 – 8 pairs, flattened above, and raised below; petiole, 1.5 – 2.5 cm long. Inflorescence, umbellate corymbs, sub terminal, bracts, and bracteoles lanceolate, deciduous. Calyx-lobes five, ovate concave, and puberulous. Corolla, cream colour violet, pink, actinmorphic, five lobed rotate shortly tubular –campanulate,obovate. Five stamens, filaments erect, basifixed anther oblong cordate; Ovary oblong One-locular, single ovule, pendulous; stigma, broadly three lobed, decurrent, subserile; Drupe, one seeded sub-globular, ribbed, crowned with persistent style; seeds compressed ventrally concave. The stem, erect, and hard; a mature stem-bark, dark brown or blackish brown with paramount irregular longitudinal fissures, and in some species a light grey coloured lichens seen. The roots even surface, hard, tortuous and pale brown with minute longitudinal fissures, root branches enormous. An altitude from 800 – 2500 ft favours the growth of *Viburnum* Linn. species, and more than one species its kind sharing a common habitat is also found.scientific informations on several species of this genus have been recorded and are approachable in number of easily accessible references[14-19].

Ayurvedic Pharmacognostical Studies

One of the noblest and holiest traditional systems of medicine to prevent and heal human ailments. Ayurvedic system of medicine originated from India and spread over several developing and developed countries. Although an artificialization in the modern system of therapy is currently very dominant, a very usage and exploitation of traditional systems of medicine are unavoidable in health practice. The modern systems of drug analysis, with or without the aid of sophisticated in-puts, is capable of formulate and standardize all existing system of medicine adopting modern scientific principles. Based on this fact it has been decided to prepare a Triple *Viburnum* churna containing the stems of three *Viburnum* Linn. species and then to standardize the same by pharmacognostical and biological means to supplement some scientific information which can be correlated with existing data base of the crude drugs.

Preparation of *V.coriaceum bark* arista by anaerobic fermentation method[20]

The barks of *V.coriaceum* were collected from Nilgiri hills, Tamil Nadu, India. A primary organic analysis on the species revealed the presence of bio-active molecules such as tannins, saponins, phenolic compounds (flavonoids) and other phenolic glycosides as their principal phyto-constituents. The crude drug (Patha) was formulated in to an arista using conventional anaerobic fermentation process for about 60 days. Apart from some traditional methods of standardization of arista, a new approach was made to select about thirteen numbers of physical, physic-chemical including organoleptic and primary organic analysis and were attempted with the arista to obtain a reproducible and consistent results and the same were recorded. The current study may act as a referential tool for how to find out novel methods of standardization of ayurvedic liquid formulations like arista and asava.

Approximately 0.612 seers (20 g) of the roots of *V.coriaceum* (patha) were coarsely powdered and added with 16 seers (512 ml of water) and boiled for about 3 – 5 h to prepare a decoction (Kashaya). The whole mixture was cooled at room temperature and filtered through a cotton cloth to obtain a decoction [20]. The decoction was taken in wooden vats of 1 litre

capacity, to which dissolved were 6.125 seers (200 g) of jaggery and boiled for half an hour. Dravyas and Dhataki pushpa (*Woodfordia fructicosa*) were then added to the mixture kept in the wooden vats. The vessel was closed with a clean lid followed by wrapping around the lid with seven consecutive layers of clay smeared cloth. The vessel was buried in cellar (basement) for about a couple of months towards the completion of fermentation process (sandhana). After the stipulated period (60 days), the vessel was withdrawn to examine the preparation which showed a brownish black fluid with a frothing and aromatic odour and alcoholic taste. The final fluid was decanted and filtered through a cotton cloth to obtain a clean transparent arista. Then the arista was bottled and labelled and subjected to some modern methods of standardization (Table 1).

Pharmacognostical Evaluation of a Triple *Viburnum* Root Churna Possessing Anti-Inflammatory Potentials[21]

Organoleptic features: The churna was yellowish brown to brown in colour with root characteristic odour, strongly astringent and slightly bitter in taste; rough and fibrous and non-mucilagenenous with water, but treatment with dilute ferric chloride solution and a mixture of phloroglucinol-HCl (1:1) turned the churna blackish and pinkish respectively. The churna under microscope showed the following components very frequently: xylem vessels with a dimension of 60 – 130 µm in diameter and most of them represented annular thickening.

The starch grains were abundant, circular, oval and semicircular single and rarely compound; they were concentric with eccentric hilum, ranging from 6 – 20 µm in size.Druses are abundant in the churna which were up to 50 µm in diameter and rarely appearing are rosette crystals.The fibres were as long as 1.2 mm in length. By employing lycopodium spore method the number of starch gains and sclereids were enumerated per mg of churna to be 1550±75 and 340±60. The percentage extractability of water and ethanol 95% v/v was determined to be 4.52±0.067 and 3.78±0.48; while crude fibre content and a loss on drying showed 10.25±0.05 and 3.85±0.035 respectively.

The percentage total ash value was determined about 3.88, being 8.52, the sulphated ash value. A notable colour change was observed under UV for alcoholic extract, treatment of powder with an acid and base, which resulted formation of reddish brown and reddish green colour respectively under long UV. In addition to observation of 80% methanolic extract under UV showing 285 nm and 235 nm as peak maxima and sub maxima, an organic analysis was carried out on the successive solvent extracts of the churna employing suitable qualitative chemical reagents. It revealed the presence of triterpenes and phytosterols in non-polar fractions and the presence of glycosides, saponins, free sugars flavonoids and amino acids with alcoholic and aqueous fractions. A spectroscopic analysis (Folin Ciocalteau, UV 650 nm) revealed the churna to possess 11.90±0.45 mg/g of total phenolic content.

The churna itself, when qualitative chemical analysis was carried with suitable chemical reagents, it gave a positive test for phenolic compounds, carbohydrates, proteins, saponins and glycosides (General). However, a chemo-microscopy of the churna was carried out under microscope revealing the presence of tanniniferous content in parenchyma of phloem; suberin in periderm cells; starch grains in medullary parenchyma; druses in cortical parenchyma and ray cells; and lignin in vascular tissue such as vessels and xylenm fibres.

The churna of *Viburnum* was subjected to preliminary phytochemical and thin layer chromatographic screenings to explore the nature of phenolics. All extracts gave a positive test for diverse classes of phenolic compounds such as tannins (Gold beater's test), chlorogenic acid (ammonia vapour treatment with ethyl acetate fraction of ethanol), flavonoids (Shinoda test), phenolic glycosides (test for reducing sugar and phenolic compounds upon hydrolysis, after exhausting free sugar) and condensed tannins (paper chromatography).

Anti-inflammatory activity

The LD_{50} was found to be more than 5000 mg/ kg b.w. p.o. in acute toxicity testing. The therapeutic dose 500mg/ kg b.w. p.o. (ED_{50}) was calculated for *in vivo* studies. The maximum effect of oedema inhibition

(Table 3) was obtained after 3rd and 5th hour of treatment at dose, the churna of 500mg/kg (27.86% and 60.34% respectively) while standard showed 38.62 % and 72.18% ($p<0.001$). Both wet and dry cotton pellets weight showed a marked reduction for the churna treated group which is significant and comparable to the standard $p<0.001$ (50.17% inhibition in case of churna treated while the standard showing 73.28%) (Table 2).

Probable mechanism of anti-inflammatory potentials of *Viburnum* root churna

Many plants, so far, have been screened to possess potent anti-oxidant property due to presence of phenolic compounds (one or more (-OH) group on the benzene moiety of their molecules). Phenolic compounds play a crucial role in counteracting excessive production and accumulation of free radicals which are powerful oxidants leading to several ailments in biological system. Formation of chronic inflammation leading to pain and other implications is a typical example of what the excessive free radicals do with healthy living being.

The receptor/ molecular level theory of a single chemical entity is experimentally predictable at ease, rather than theory of drug mechanism for a crude extract. Plant extract may possess indefinite number of components, which on administration in a living system may target a wide range of receptors or their relevant factors at a single time point, or every single component of the extract may target not more than one receptor type at a single time point, which leads to an increase in magnitude of the drug activity. Considering this phenomenon in to account, a probable mechanisms of action of churna is possibly unfolded in this study which deserves a mention and can be useful to progress advanced pharmacological studies.

The mechanism of biological activity of extracts *in vivo* may be probably through one or all of the following ways: Phospholipid metabolism is catalysed by enzymes such as phospholipase A_2 (PLA_2), cyclooxygenase ($COX_{1,\ 2\ and\ 3}$), lipooxygenase (LO, 5LO, 12LO and 15LO) and acetyl transferase (AT) that leads to formation of various inflammatory mediators such as prostaglandins (prostanoids) (PGI_2, $PGE_{2\alpha}$, PGD_2 and PGE_2), thromboxanes (TXA_2), leukotrienes (LTA_4, LTC_4 and LTB_4) and platelet activating factor (PAF).

The phenolic compounds of the extracts, especially flavon-3-ols, biflavones, flavonones of flavonoids classes, both in free and glycoside form, possess a potent anti-inflammatory activity by targeting COX, LO and AT by forming phenol-proteins complex leading to blockade of their action thereby preventing conversion of metabolites from their precursors to form the inflammatory mediators. The enzyme deactivation may be either reversible or irreversible is a matter of speculation, which can be proven only over some advanced studies involving the receptors. It is also probable that unlike conventional NSAIDs the extracts contain several phenolic compounds of diverse chemical structure which may target amino acid domains of the COX by hydrogen bonding to subside the functional status of the enzyme.

Formulation of *Viburnum Erubescens* Root Asava and its Physico-Chemical Standardization[22]

The results of physical and physic-chemical analysis of *V.erubescens* root asava were tabulated and discussed in detail under the section discussion. The primary organic analysis on the both ethanolic extract of the crude drug (Patha) as well as the asava itself gave a positive test for carbohydrates (Molisch's test); amino acid (Xanthoproteic test); free sugar (Fehling's' and Benedict's test); tannins (Gold beater's test); general phenolic compounds (dilute ferric chloride test); flavonoid (Shinoda's test and pH dependent colour test by Mg-HCl); saponins (Haemolytic test); general glycosides (by hydrolytic test after exhausting free sugar); phenolic glycoside (by hydrolysis followed by phase separation by non-polar solvent and testing of the same); and the presence of anthocyanins (Blood red colouration of both alcoholic and aqueous extract). An organoleptic analysis was also carried out on the asava. The *V.erubescens* root asava itself and the asava added with water, 80% methanol and ethylacetate were observed under UV radiation showing dark brown, yellowish brown, yellowish brown and pale brown colouration respectively. A primary organic analysis conducted on the asava itself as well as the ethanolic extract of the patha revealed the presence of carbohydrate, amino acid, free sugar,

saponins, tannins, phenolic compounds (general), flavonoids, saponins and glycosides (phenolic glycosides). However, presence of phyto-sterols and triterpenes were in the negative. The asava was brownish black in colour; aromatic in odour; aromatic and sweet in taste; sticky after minutes in texture between fingers; pourable and slightlysticky in nature to view; it showed a darkening after its evaporation, when kept under room temperature; and smelled ethanolic and pleasant while heating on a boiling water bath.

The term total solid is applied to the residue obtained where the prescribed amount of the preparation is dried to constant weight. The total solid of the asava were determined to be 46.6±0.2% w/ml. The lower limit of the range is the temperature indicated by the thermometer when the first drop of condensate leaves the tip of the condenser and the upper limit is the temperature at which the last drop evaporate from the lowest point in the distillation flask, as far as distilling range of the asava is concerned. In this event, the asava showed 72±0.02° C to 106±0.08° C as its boiling range.

The congealing temperature is that point at which there exists a mixture of the liquid (fused) phase of a substance and increasing proportion of solid phase. The asava, in this case, showed 63±0.08° C to 66±0.04° C as the congealing point. Making no modification in the setting of apparatus the freezing point of the asavta was determined to be 8±0.06° C. Since the principle behind the formulation of asava is that conversion of sugar (jaggery) in to ethanol by anaerobic fermentation process, the total alcohol concentration was determined to be 21% v/v at 32° C by distillation cum specific gravity method. Loss on drying is a versatile method of standardization applicable for materials existing in liquid, solid, semisolid state. On the basis of the above principle, loss on drying of the asava was determined to be 22.42±0.60% w/w.

Although loss on ignition is best suiting to standardize formulation such as churna, it cannot be stated that asava may not be standardizable by this method. Because, the principle behind the loss on ignition is to determine the quantity of inorganic elements which could be convertible in to their corresponding oxides, which include both physiological as well as non-physiological ashes. Hence, the loss on ignition of the asava in percentage w/v as determined to be 2.9±0.33% w/v. To determine the acidity or alkalinity of the asava, pH value was determined to be 4.4 by potentiometric method. Determination of refractive index is one of the best suiting standardizing process for liquid formulation with reference to air; the refractive index of the asava using as Abbe refractometer against water was measured to be 1.499. By employing an Oswald - type viscometer, viscosity was determined against water to be 1.8772 poise at 32°C. Since asava is a liquid formulation, by using a calibrated Pygnometer, the weight per ml of the asava was determined to be 1.055 g/ml at room temperature. The total free sugar content using Benedict's reagent for quantitative analysis was determined to be 25 g %.

***Viburnum* species for its pharmacognosy**[23]

The leaves, stems and the roots of *Viburnum punctatum* Buch.-Ham. ex D.Don, *Viburnum coriaceum* Blume and *Viburnum erubescens* Wall. ex DC. were collected from Nilgiri and Coimbatore hills, Tamilnadu, India. Transverse sections (TS) of plant roots were prepared with aid of a rotary microtome. The TS of 10 µm thickness were stained and fixed in Canada balsam and morpho-anatomical features of each specimen were noted. The specimens were powdered using a mechanical grinder and were mounted in suitable media for micro-measurements employing a swift Ives' camera lucida and a calibrated eye piece micrometer. This study resulted to obtain very consistent and reproducible parameters, which help differentiating the three species based on their micro-morphology and micro-measurements.

Biological Potential of *Viburnum* Species

Total phenolics and anti-oxidant potentials of *Viburnum* species[24]

The stems of *Viburnum punctatum*, *Viburnum coriaceum* and *Viburnum erubescens* were shade dried for a week. About 500 g of powdered samples were extracted with petroleum ether (60-80 °C), benzene and 75 % (v/v) ethanol successively in a soxhlator one by one

and followed by determination of percentage extractives. The extracts were qualitatively tested for different chemical constituents present. The ethanolic extracts were selected for further investigations, such as total phenolic content and *in vitro* antioxidant potential. It was revealed that all the three species exhibited a significant antioxidant potential against the reference compounds subjected and antioxidant potential among three species was ascending in series: *V. coriaceum* > *V. punctatum* > *V. erubescens*.

Anti inflammatory and tocolytic potentials of three *Viburnum* species[25]

The current study undertaken casts some scientific information on phytochemical based pharmacological screening such as anti-inflammatory, analgesic and anti-spasmodic activities, using ethanolic stem extracts of three species – *Viburnum punctatum* (VPEE), *Viburnum coriaceum* (VCEE) and *Viburnum erubescens* (VEEE) – in suitable animal models. In carrageenin induced paw oedema (in rats), (VPEE, VCEE and VEEE each of 500 mg/ kg b.w) showed a significant reduction in paw oedema volume ($p<0.001$) and was comparable to the standard (Indomethacin 10mg/kg b.w). In cotton pellet induced granulation method in wistar rats, a significant reduction in dry weight and wet weight of cotton pellets was observed with all extracts ($p<0.001$) which was comparable to the standard (Indomethacin 20mg/kg b.w). In acetic acid induced writhing in mice, a significant reduction in writhing was observed with all extracts than control ($P<0.001$). However, reduction in number of writhings was not proximal to Diclofenac sodium 15 mg/kg b.w. Many plants, so far, have been screened to possess potent anti-oxidant property due to presence of phenolic compounds (one or more (-OH) group on the benzene moiety of their molecules). Phenolic compounds play a crucial role in counteracting excessive production and accumulation of free radicals which are powerful oxidants leading to several ailments in biological system. Formation of chronic inflammation leading to pain and other implications is a typical example of what the excessive free radicals do with healthy living being. The receptor/molecular level theory of a single chemical entity is experimentally predictable at ease, rather than theory of drug mechanism for a crude extract. Plant extract may possess indefinite number of components, which on administration in a living system may target a wide range of receptors or their relevant factors at a single time point, or every single component of the extract may target not more than one receptor type at a single time point, which leads to an increase in magnitude of the drug activity. Considering this phenomenon in to account, a probable mechanisms of action of VPEE, VCEE and VEEE is possibly unfolded in this study which deserves a mention and can be useful to progress advanced pharmacological studies. The mechanism of biological activity of extracts *in vivo* may be probably through one or all of the following ways: Phospholipid metabolism is catalysed by enzymes such as phospholipase A2 (PLA2), cyclooxygenase (COX1, 2 and 3), lipooxigenase (LO, 5LO, 12LO and 15LO) and acetyl transferase (AT) that leads to formation of various inflammatory mediators such as prostaglandins (prostanoids) (PGI2, PGE2α, PGD2 and PGE2), throboxanes (TXA2), leukotrienes (LTA4, LTC4 and LTB4) and platelet activating factor (PAF).

The phenolic compounds of the extracts, especially flavon-3-ols, biflavones, flavonones of flavonoids classes, both in free and glycoside form, possess a potent anti-inflammatory activity by targeting COX, LO and AT by forming phenol-proteins complexleading to blockade of their action thereby preventing conversion of metabolites from their precursors to form the inflammatory mediators. The enzyme deactivation may be either reversible or irreversible is a matter of speculation, which can be proven only over some advanced studies involving the receptors. It is also probable that unlike conventional NSAIDs the extracts contain several phenolic compounds of diverse chemical structure which may target amino acid domains of the COX by hydrogen bonding to subside the functional status of the enzyme. Anti-spasmodic and tocolytic effects of the test drugs *in vitro* may be that the GIT is provided with diverse number of chemical mediators and their respective receptors such as muscarinic (M3), tryptaminergic (5HT2α and 5HT4) histaminic (H1 and H2), nor-adrenergic (α1, α2 and β2) and prostanoid receptors (DP, EP for PGD2 and PGEP1,2). Intestinal spasm is triggered by histamine and acetylcholine is due to agonistic activity on H1 and M3 receptors located therewith respectively. The extract in combination with histamine, and then with acetylcholine showed a decrease in contractility of ileum was the

observation in the current study. The test drugs at the dose level of 64, 32 and 128; and 32, 16 and 64 mg/ml were able to exhibit a complete blockade of agonistic activity of histamine and acetylcholine. Therefore, it may be concluded that the components of extracts competitively bind with H1 and M3 population of ileum tissue and thereby produce an antagonistic effect which seems to be atropine like and antihistaminic-like activities. The uterine muscles (endometrium and myometrium) are provided with various receptors of various chemical mediators such as: histaminic (H1 receptors), oxytocic receptor, adrenergic (α, β2 receptors), tryptaminergic (5-HT receptors), and prostanoid receptors (DP, FP, IP, TP and EP receptors to accommodate PGI2, PGE2α, PGD2 and PGE2 respectively). The phytoconstituents of VPEE, VCEE and VEEE, if antagonistic to any of these receptors or their relevant factors, may cause a relaxation of uterine muscle. In some literatures it has also been noted that the bark extracts of some *Viburnum* Linn. Species exhibited uterine sedative effect which is beneficial especially during the complications such as dysmenorrhoea and menorrhagia in the non-pregnant uterus. Etiology of these complications is attributed to the excessive production and activity of PGE2, PGF2α and PGI2 induced by an imbalance in sex hormones in systemic circulation and their activities. It is likely that the chemical molecule which can imitate the action of NSAIDs, when administered exogenously, may provide a relief from these complications.

Based on the above fact, aspirin induced tocolytic potential against oxytocin-induced uterine contraction was selected as a reference in the current study. The ability of test drugs to act against oxytocin-induced contractions to cause relaxation was undertaken as an appropriate experimental set. The test drugs being potential anti-inflammatory in action caused a relaxation of uterine horns, which was comparable to that of conventional NSAIDs. Hence, it may be concluded that the components of all the three extracts distract oxytocin induced prostanoid mediated contractility in myometrium and endometrium region of the uterus by targeting FP, TP, IP and EP3 receptors of prostanoids present therewith. In consequence, dilation in uterine muscle tone is incident, which is also comparable to aspirin induced tocolytic effect.

Antiulcer activity of three *Viburnum* species[26]

The stem parts of these species claim to contain an appreciable quantity of therapeutically valuable phenolic compounds like anthocyanins, phenolic acids, flavones, flavonols and biflavone, and their glycosides. A few of them have been reported to exhibit uterine sedative, antiasthamtic, anti-oxidant, astringent, anti-spasmodic and antimicrobial activities. Based on the above facts on records, the present study has been under taken with an objective of screening the antiulcer potentials o 75% v/v aqueous ethanolic stem extracts of some three species of this genus, namely: *V.punctatum*, *V.coriaceum* and *V.erubescens* by aspirin plus pylorus ligation model in rats, using Ranitidine 50 mg/ kg b.w (p.o) as the positive reference drug. From the findings of ulcer score, histo-pathological features and the status of biochemical parameters of gastric contents, it is concluded that extract of *V.coriaceum* at a dose level of 500 mg/kg.b.w possessed a significant antiulcer activity ($p<0.01$, $p<0.001$). However, the magnitude of antiulcer potential among the species was not far different.

In this event, the constituents of test drugs are supposed to block muscarinic receptors of the oxyntic cells leading to a partial blockade in the release of histamine followed by a suppression of H2 dependent acid formation and its release to the lumen of the stomach. Phenolic molecules are efficient reactors prone to bind with proteins and thereby leading to denaturation (often called astringent effect). Among phenolic compounds, the ones, namely: tannins – a powerful astringent. The VPEE, VCEE and VEEE were confirmed to contain tannins of condensed nature and their relevant polymeric compounds such as procyanidins and anthocyanidins (blood red colour – brownish nature of alcoholic and aqueous extracts on heating). So, it may be a way that the tannins of test drugs can afford an astringent activity on the mucosal and sub-mucosal part and thereby resisting further corrosions of stomach walls by acid and also progress a healing process in the lumen.

Prepapration of Triple *Viburnum* churna[27]

The authenticated samples were, carefully examined with the aid of a dissection microscope for the presence of foreign matter, which yielded not more than 0.252% of contaminants. About 50 g each of stems of VP, VC and VC were dried in the sun for 15 days and powdered using a mortar and pestle after mechanically ground in to coarse powder. The powders (1:1:1 ratio) were passed through muslin cloth. And added were 150 g of Jaggery (1:1 ratio) and triturated well and stored in an air tight container to prevent the entry of moisture and other contaminants. The churna was subjected to several physic-chemical and micrometric analysis and the results were recorded.

Anti-inflammatory activity of *Viburnum* churna

Triple *Viburnum* churna containing the stems of three *Viburnum* Linn. species was prepared and then standardized the same by pharmacognostical and biological means to supplement some scientific informations which can be correlated with existing data base of the crude drugs. So, the current study undertaken is to cast some scientific information on formulation based pharmacological screening of a crude drug for its anti-inflammatory activity, using a combined churna prepared from the stems of three species – *Viburnum punctatum* (VP), *Viburnum coriaceum* (VC) and *Viburnum erubescens* (VE) – in suitable animal models. Firstly the churna was evaluated pharmacognostically to obtain reliable and reproducible parameters. Then, the biological screening was progressed with determination of LD_{50} value to ensure a safety of the animal experimentation.

In carrageenan induced paw oedema (in rats), (a mixed churna of stem of VP, VC and VE of 500 mg/ kg b.w) showed a significant reduction in paw oedema volume ($p<0.001$) and was comparable to the standard (Indomethacin 10mg/kg b.w). In cotton pellet induced granulation method in wistar rats, a significant reduction in dry weight and wet weight of cotton pellets was observed with the churna ($p<0.001$) which was comparable to the standard (Indomethacin 20mg/kg b.w)(Table 4).

Anti-diarrhoeal activity of *Viburnum* root asava[28]

A primary organic analysis on the species revealed the presence of bio-active molecules such as tannins, saponins, phenolic compounds (flavonoids) and other phenolic glycosides as their principal phyto-constituents. The crude drug (Patha) was formulated in to an asava using conventional anaerobic fermentation process for about 90 days. Apart from some traditional methods of standardization of asava, a new approach was made to select about thirteen numbers of physical, physic-chemical including organoleptic and primary organic analysis and were attempted with the asava to obtain a reproducible and consistent results and the same were recorded. Since *V.coriaceum* root asava contained phenolic compounds as principal constituents, a screening for its anti-diarrhoeal potential was attempted. The asava showed a markable and significant anti diarrhoeal activity against Castor oil induced diarrhoea.

Probable mechanism of anti-diarrhoeal activity of *Viburnum* asava

The oral administration of castor oil is acid hydrolysed in the gastrointestinal tract in to recinoleic acid; the later is converted in to its salt form namely: sodium recinoleate which is a powerful irritant of mucosal membrames of the physiological system. The irritant effect of sodium recinoleate increases the propulsive or peristaltic movement leading to drastic purgation.

The *Viburnum* asava has been analysed to contain tannins and other phenolic compounds which may accelerate astringent effect (precipitation of mucosal proteins). Hence, there has been a formation of a barrier which resists the irritant effect of castor oil and its further effect on the GIT inducing diarrhoea.

Antiulcer activity of *Viburnum* arista[29]

A primary orgnic analysis was undertaken on the various solvent extracts of increasing polarity, which revealed that the polar and moderately polar extracts showing a positive test for phyto-sterols, triterpenes and hydroalcohloic and aqueous extracts representing the presence of saponins, carbohydrate (sugars), amino acids and well pronouncing phenolic compounds such as tannins, flavonoids and phenolic glycosides.

In water immersion plus restraint stress ulcer method, there were three groups of animals. The severity index of bleeding was significantly increased ($p<0.001$) in the group-II animals as positive control, when compared to control group-I. Administration of arista equivalent to and 200 mg/kg b.w to group-III showed a significant ($P<0.001$) decrease in ulcer severity index when compared to the positive control.

The ulcer score was significantly increased ($P<0.001$) in the group-II animals, when compared to that of the control (group-I). Administration of arista equivalent to 200mg/kg b.w treated groups (III) showed a significant ($P<0.001$) decrease in ulcer score, when compared to group-II.

The biochemical factors, which are relevant to WIRS experimentation, were screened with all groups of rats revealed the following facts: Status of SOD in ulcer was determined using a spectrophotometer at 480 nm and reported in terms of unit activity (1 unit SOD activity is the amount of enzyme required to give 50% inhibition of epinephrine auto-oxidation). Superoxide dismutase level was significantly increased ($p<0.001$) in untreated group, when compared to control group. Treatment with ethanolic extract of arista equivalent to 200 mg/kg dose showed a significant decrease ($p<0.001$) in SOD level in stomach homogenate, when compared to that of the control.

Catalase level was significantly ($p<0.001$) reduced in solvent control group (group-II), when compared to that of the normal control group (group-I). Arista equivalent to at 200 mg/kg b.w dose showed a significant ($p<0.05$) increase in CAT levels in stomach homogenate, when compared to that of the control.

Lipid peroxidation in stomach homogenate was significantly increased ($p<0.001$) in untreated group, when compared to control group. Treatment with arista equivalent to 200 mg/kg dose showed a significant decrease ($p<0.001$) in LPO level in stomach homogenate, when compared to that of the control (group–II). The gastric wall mucous content represented as absorbance OD, was significantly decreased ($P<0.05$) in the group-II animals when compared to control group-I. Administration of arista equivalent to 200 mg/kg showed significant ($P<0.05$) increase in GWMC when compared to that of the solvent control.

Histopathological observation of WIRS ulcer stomach

Histopathological changes occurred in the stomach of experimental animals of different groups were shown in (Figure 1). Group I (normal control) showed a physiologically normal histology; group II (positive control) showed distinct superficial erosions. The group dosed by arista represented physiologically healthy mucous gland, which is comparable or greater than that of the group I.

Probable mechanism of actions behind the biological activities of *V. coriaceum* root arista

The ayurvedic formulation – Arista was prepared based on the mechanism "Alcohol formed sugar by fermentation process extracts all phenolic compounds" out of crude drug and makes available for physiological action. Many plants, so far, have been screened to possess potent anti-oxidant property due to presence of phenolic compounds (one or more (-OH) group on the benzene moiety of their molecules). Phenolic compounds play a crucial role in counteracting excessive production and accumulation of free radicals which are powerful oxidants leading to several ailments in biological system. Formation of chronic inflammation leads to a non-relievable pain and pain relevant implications is a typical example of what the excessive free radicals do with living beings

The receptor/ molecular level theory of a single chemical entity is experimentally predictable at an ease, rather than a theory of drug mechanism for a crude extract. A medicinal plant extract may possess several chemical components, which on administration in a living system may target only one kind of receptor at a single time point (i.e., all to one) or more than one receptor type at a single time point. Considering this phenomenon, a probable mechanism of action of VPEE, VCEE and VEEE are unfolded in this study which may be useful to progress some advanced pharmacological studies on these species in future.

Mechanism of action

Ulceration by WIRS model is based on the principle- "stress induced pathophysiological occurrence". Vagus

nerve (10th parasympathetic nerve originated from the cranial outflow) is innervated to most of the visceral organs to control over diverse number of physiological function.

When the animals are allowed to swim in water for a long time, a stress is created leading to the induction of unbalanced vagus tone in GIT, which induces Ach leading to activation of M_3 receptors. Then, the sequence of incidences proceeds aggravation of ulceration. The muscarinic receptors of oxyntic or parietal cells posses tendency to activate mast cells, which are neighbouring receptors to (M_3), to augment the release of histamine which leads to an activation of (H_2) histamine-2 dependent K^+/H^+–ATPase pump.

In this event, the constituents of test drugs are supposed to block muscarinic receptors of the oxyntic cells leading to a partial blockade in the release of histamine followed by a suppression of H_2 dependent acid formation and its release to the lumen of the stomach. So, it may be a way that the tannins of test drugs can afford an astringent activity on the mucosal and sub-mucosal part and thereby resisting further corrosions of stomach walls by excessive acid and also progress and promote a healing process in the lumen.

Antiulcer activity of Ethanolic leaf extracts of three *Viburnum* linn. Species – a Comparative evaluation[30]

VCEE, VEEE and VPEE were screened for their antiulcer potential at a dose level of each 500 mg/kg b.w, p.o and the result of the sudy as follows: There were six groups of animals subjected in aspirin plus pylorus ligation model. The ulcer score was significantly increased ($p<0.001$) in the group-II animals, when compared to control group-I. Administration of VCEE, VEEE, VPEE and Ranitidine showed a significant ($p<0.001$) decrease in ulcer score, when compared to group-II. With aid of a pH meter, the pH of the stomach content was monitored; the pH level was significantly increased ($p<0.001$) in the group-II animals as positive control, when compared to that of the solvent control (group-I); and administration of VCEE, VEEE, VPEE to group-III, IV & V respectively, and Ranitidine-dosed (group-VI) animals showed a significant ($p<0.001$) decrease in pH level, when compared to group-II animals.The gastric volume was significantly increased ($P<0.001$) in the group-II animals, when compared to control group-I. Administration of VCEE, VEEE, VPEE and Ranitidine showed a significant ($P<0.001$) decrease in gastric volume level (III, IV and V groups), when compared to that of the group-II.The free acidity was significantly increased ($P<0.001$) in the group-II animals, when compared to control group-I. Administration of VCEE, VEEE, VPEE and Ranitidine showed a significant ($P<0.001$) decrease in free acidity, when compared to that of the group-II. The total acidity was significantly increased ($P<0.001$) in the group-II animals, when compared to control (group-I). Administration of VCEE, VEEE, VPEE and Ranitidine showed a significant ($P<0.001$) decrease in total acidity, when compared to that of the group-II. The total protein was significantly increased ($p<0.001$) in the group-II animals, when compared to control group-I. Administration of VCEE, VEEE, VPEE and Ranitidine showed a significant ($p<0.001$) decrease in total protein level, when compared to that of the group - II animals.The total hexose was significantly decreased ($p<0.001$) in the group-II animals, when compared to control group-I. Administration of VCEE, VEEE, VPEE and Ranitidine showed a significant ($p<0.001$) increase in total hexose, when compared to that of the group-II).The hexosamine was significantly decreased ($p<0.001$) in the group-II animals, when compared to control group-I. Administration of VCEE, VEEE, VPEE and Ranitidine showed a significant ($p<0.001$) increase in hexosamine, when compared to group-II. The fucose was significantly decreased ($p<0.001$) in the group-II animals, when compared to that of the control (group-I). Administration of VCEE, VEEE, VPEE and Ranitidine showed a significant ($p<0.001$) increase in fucose, when compared to that of the group-II .The total carbohydrate was significantly decreased ($p<0.001$) in the group-II animals, when compared to that of the control (group-I). Administration of VCEE, VEEE, VPEE and Ranitidine showed a significant ($P<0.001$) increase in total carbohydrate level, when compared to group-II.The sialic acid was significantly decreased ($p<0.001$) in the group-II animals, when compared to that of the control (group-I). Administration of VCEE, VEEE, VPEE and Ranitidine level showed a significant ($p<0.001$) increase in sialic acid, when compared to that of the group-II.The status of biochemical parameters were

comparable to the solvent control as well as the reference drug-treated group which is shown in the glandular portion and its pathological symptoms.

Ulcer induction and aggravation in aspirin plus pylorus ligation model and its counteraction by VCEE, VEEE and VPEE

Acetylsalicylic acid, itself, is an acid which reduces a rise in pH of the GIT. Moreover, aspirin can block the harmonious supply of HCO_3^- ions, which are supplied from the blood stream through the vascular bed of the stomach. This is governed by the effect of a prostaglandin. A failure in supply of HCO_3^- ions leads to a rise in acid accumulation.

Probable mechanism of action of VPEE, VCEE, VEEE against aspirin induced and acid accumulation upon ligation of pylorus

All the extracts have, already, been proven to be anti-cholinergic in action (especially, muscarinic blocker).The muscarinic receptors of oxyntic or parietal cells posses tendency to activate mast cells, which are neighbouring receptors to (M_3), to augment the release of histamine which leads to an activation of (H_2) histamine-2 dependent K^+/H^+–ATPase pump.In this event, the constituents of test drugs are supposed to block muscarinic receptors of the oxyntic cells leading to a partial blockade in the release of histamine followed by a suppression of H_2 dependent acid formation and its release to the lumen of the stomach. Phenolic molecules are efficient reactors prone to bind with proteins and thereby leading to denaturation of protons (often called astringent effect). Among phenolic compounds, the ones, namely: tannins – a powerful astringent. The VPEE, VCEE and VEEE were confirmed to contain tannins of condensed in nature and their relevant polymeric compounds such as procyanidins and anthocyanidins (blood red colour – brownish nature of alcoholic and aqueous etracts of these species on heating).So, it may be a way that the tannins of test drugs can afford an astringent activity on the mucosal and sub-mucosal part and thereby resisting further corrosions of stomach walls by excessive acid and also progress and promote a healing process in the lumen.

Phytochemical Investigations on *Viburnum* Species: Total Phenolic Content and *In vitro* Anti-oxidant Potentials of Ethanolic Root Extracts of Three *Viburnum* Linn. species – A Comparative Study[31]

Primary organic analysis

A qualitative chemical examination performed on the roots of *V.punctatum*, *V.coriaceum* and *V.erubescens* revealed the presence of phytosterols and triterpenoids in petroleum ether and benzene fractions; triterpenoids in chloroform fraction; glycosides (phenolic) and phenolic compounds and reducing sugar in alcoholic and aqueous fractions. After exhausting free sugars, the extracts were investigated for the chemical nature of non-sugar (aglycone) part of their glycosides; and result was in favour of phenolic compounds.

Total phenolic

Confirming the presence of phenolic compounds such as tannins andflavonyl glycosides, it was decided to screen the total phenolic content in terms of catechol equivalent w/w, it was also revealed that the percentage phenolics from VCEE was higher than that of VPEE and VEEE. But the percentage deviation among the three species was not assortable quantitatively, VC: 11.0 $\pm$ 0.52 mg/g; VE: 8.5.5 $\pm$ 0.42 mg/g and VP: 10.5 $\pm$ 0.50 mg/g.

***In vitro* anti-oxidant potential of the *Viburnum* Linn. Species**

The basic principle of this analysis is that the dose dependant capacity of the test samples to scavenge DPPH radicals against a standard chemical agent that works in the similar way, a typical example is, Vitamin-C (Ascorbic acid) which is one of the popular and potent anti-oxidants.Then, such three more parameters were also selected to assess the anti-oxidant potentials of the test samples such as nitric oxide scavenging, hydroxyl radical scavenging and reducing power), preferring vitamin-E and BHT (Butylated Hydroxy Toluene) as the references with aid of a spectrophotometer. The report was presented in terms of IC_{50} (Inhibitory Concentration) values. VCEE, VEEE and VPEE were observed to scavenge DPPH radicals equally until 200 µg dose level when compared to that of the standard. However, at concentration, 800 µg, vitamin-C overwhelms the capacity in scavenging DPPH

radicals.The measurement range being 68.51±1.2903, 65.43±1.350 and 62.36±1.203 for VCEE, VEEE and VPEE at 800 µg, against the standard, vitamin-C which assumed about 80.62±0.270. However, IC_{50} values in µg between the tests samples and the references were not markably distinct. In case of nitric oxide scavenging activity, the standard, vitamin-E, showed 218.4±1.33 as IC_{50} value, while VCEE, VEEE and VPEE being 300.68±2.0, 323.4±2.3 and 311.43±2.1 respectively.In this case,VCEE showed a dominant effect.

Hydroxyl radical scavenging tendency of the standard, from 25 – 800 µg dose level, was far more rising, when compared to that of test samples at the same concentrations. However, among three species, VPEE showed,255.90±0.52, a significant OH^- radical scavenging activity when compared to other two species(405.15±1.42,302.55±0.83).

The standard BHT, at 700 nm, at various dose (Concentration) levels, 125, 250, 375 and 500 µg, showed a gradual increase in absorbance; in the same way, all three samples afforded significant reducing power, which was comparable to the reducing power of the reference. BHT being,0.981±0.041, while VCEE, VEEE and VPEE showing 0.943±0.039,0.602±0.047 and 0.924±0.087 respectively as their absorbance. In this case, VCEE represented a dominant activity.

Histo-Chemical Investigation on the Leaves, Stem and the Roots of Three Viburnum Species[32]

A histo-chemical analysis was executed to locate and find out the phyto-constituents of three *Viburnum* Linn. species (Table 6.).

A Coparative preliminary phytochemical screening on the leaves, stems and the roots of three *Viburnum* Linn.species[33]

The percentage extractive of leaves was higher than that of the stem and roots, 7.08±0.064 (aqueous), in case of *V.punctatum*. However, the yield by ethanol (75% v/v) of the leaves, stem and root were proximal to each other being, 3.21±0.64, 2.32±0.106 and 2.48±0.161 respectively. In case of *V.coriaceum*, aqueous extract of leaves showed the highest percentage extractives (6.53±0.264) followed by 75% v/v aqueous ethanolic extract (3.46±0.201, 3.12±0.211 and 2.88±0.167 for leaves, stems and roots respectively). *V.erubescens* exhibited unusual results that the percentage extractives by ethanol 75% v/v were higher than that of any other solvents especially, the aqueous fraction, 5.93±0.067, in case of leaves and roots. Petroleum ether (60 – 80°C), benzene and chloroform fractions of leaves of VP revealed the presence of sterols and triterpenes, while alcoholic and aqueous fractions gave a positive test to free reducing sugars, glycosides, saponin and some phenolic compounds. The stem part of the species showed the presence of triterpene, sterols in chloroform and ethereal fration, while phenolic compounds were observed very distinctly in the alcoholic fraction.

The root of the species gave a positive test for phenolic compounds and their glycosides with ethanolic and aqueous fraction, while chloroform and ethereal layers showed the presence of triterpenes. The leaves showed the presence of phyto-sterols, and triterpene in pet.ether, chloroform and benzene fractions, while phenolic compounds and their glycosides were positive with aqueous fraction. However, 75% alcoholic fraction was showing glycosides to an ignorable extent. The stem gave a positive test for triterpenes in its ethereal and chloroform layer, while 75% alcohol showing a more pronounced results for the presence of phenolic compounds and their glycosides. Nevertheless the results of the tests for triterpenes were also equally distinct. The root of the species showed no any remarkable result when compared to that of the first species (VP) However, the presence of phyto-sterols in benzene was found rather than in ethereal, and in chloroform-fractions.

V.erubescens was screened for diverse classes of its constituents, subjecting their successive solvent extracts revealed a very limited diversity from its co-species, VP and VC. The stem extracts of VE witnessed a more pronounced results for the presence of triterpenes rather than phyto-sterols in their non-polar fractions, the results were very similar to that of Viburnum punctatum. In addition to the above, 75% alcoholic stem extract also exhibited the presence of phenolic glycosides, which was confirmed by exhausting the free sugars from the alcoholic extract follwed by an acid

hydrolysis using M/2 HCl on a boiling water bath for about 30 min.

A Preliminary Chromatographic Detection of Phenolic Compounds from Ethanolic Stem Extracts of *Viburnum* Linn.Species by TLC And PC[34]

TLC

The R_f value of each reference compound was recorded by ascending TLC as performed for the test samples. VPEE exhibited 4 spots, A fluorescence quenching zone under UV-254 nm; An intensive orange and a yellow fluorescence were found after spraying PEG-NP reagent on the chromatogram and then observed under UV-365 nm;Rf values resulted by test samples of VP, were 0.95, 0.70; for VC, 0.9; and in case of VE, 0.45, 0.40 which were parallel to the R_f values of reference compounds, astragalin, rutin, ferulic acid, chlorogenic acid and caffeic acid.

Detection of arbutin by TLC

Detection by locating agent was carried out using three types of reagents separately.

Berlin blue reagent (BB)

When 5-10 ml of BB were sprayed on the plate, a blue zone appeared at R_f value 0.40 of test solution, which was comparable/ parallel to the R_f value of reference compound mixture a blue zone against day light.

NP-PEG reagent

Yellow brown zones were seen in UV-365 nm, whose R_f value (0.40)

Potassium hydroxide reagent (8 – 10% ethanolic KOH)

In UV-365 nm, yellow-green fluorescent zone appeared whose R_f value (0.40).

Descending paper chromatography for detection of rutin

The paper chromatogram under long UV-365 nm, showed a pale yellow spot (R_f value: 0.15, 0.45 and 0.83) when mobile phases 1, 2 and 3 were used. After a spray of 1% aluminium chloride solution over the paper chromatogram showed a distinct greenish yellow spot in case of VP.

Mobile phases

Ethyl acetate saturated with water; Phenol saturated with water;60% isopropanol and 40% water mixer.

Detection of condensed tannins

Paper chromatography (two dimensional and ascending)

About 100 mg each of ethanolic stem residue of VP, VC and VE were treated separately with 5 ml of 50% methanol (λ_{max} 475 and 560 nm visible region) and filtered. 30 – 40 µl of the filterates were spotted on Whatmann filter paper No.1 with aid of a micropipette. The paper was subjected to two dimensional chromatography using (Butane-2-ol : Acetic acid : Water) at ratio 14:1:5 followed by 6% acetic acid as developing phase. The paper chromatogram was air dried and exposed to UV-254 nm, which showed dark purple spots indicating the presence of condensed tannins in all three species.

Detection of amentoflavone by TLC

The thin layer chromatograms of VP, VC and VE spotted were developed using chloroform-acetone-formic acid (75:16.5:8.5) as developing phase. The chromatogram of VC showed a distinct fluorescent orange spot (R_f-0.74) upon a spray of KOH reagent under long UV-365 nm. The characteristics spots on the chromatograms of the individual species show the probable presence of the following phenolic compounds: **VP stem extract:** astragalin, rutin and arbutin; **VC stem extract:** amentoflavone, caffeic acid and arbutin; **VE stem extract:** chlorogenic acid and rutin. Rutin – A flavonol glycoside is a common constituent of VP and VE, while arbutin with VP and VC. But, amentoflavone, astragalin and chlorogenic acid are not evident as that of the formers. However, the condensed tannins are not species specific (i.e, found in all the three species).This study can be a referential tool for progressing isolation of these phnolic compounds from the species subjected in this study by trial and error methods.

Isolation of phenolic compounds from *Viburnum* species

TLC detection of phenolic compounds

A chromatographic detection was attempted with the phenolic compounds such as flavonoids, phenolic acids,

hydroquinone derivatives, biflavone and condensed tannins. Ascending chromatogram was developed using silicagel-G (TLC grade) and when required, silicagel $60F_{254}$ (Merck) as stationary phases, appropriate solvent or solvent combinations as developing phase. The sample spots on the chromatogram were detected under short UV, 254 nm, and long UV, 365 nm. Many of these species have been reported to possess phenolic compounds and their glycosides, in addition to sterols and triterpenes, as their major chemical constituents in the stem and root barks. These chemical constituents may be attributed to their remarkable biological activities against smooth muscle related troubles. The main objective of the present study is to detect, isolate and characterize some phenolic compounds from aqueous ethanolic stem extract of two *Viburnum* Linn. species, collected from Nilgiri hills, Tamilnadu, India. The dried stems of *Viburnum punctatum* Buch.-Ham.ex D.Don and *Viburnum coriaceum* Blume. were powdered and extracted separately, successively with petroleum ether (60 – 80°C), chloroform, 75% aqueous ethanol. The ethanolic extracts were screened by Thin Layer Chromatographic techniques for the presence of different classes of phenolic compounds, since the glycosides of phenolic compounds such as flavones, flavonols, biflavone and phenolic compounds of C_6-C_3 are appreciably soluble in alcohol rather than the other two fractions. The TLC studies revealed the presence of some flavonoids which was later confirmed by spectroscopic analysis and melting point to be astragalin in *V.punctatum* and amentoflavone in *V.coriaceum*. The ethanolic residues of *V.punctatum* and *V.coriaceum* were adsorbed on the activated neutral alumina and packed in a glass column containing slurry of alumina in petroleum ether. Then, the column was eluted firstly with some non-polar and moderately polar solvents to clear out impurities, which remain intact in the column. Then, the content of the column was subjected to a gradient elution with methanol-water mixture of decreasing polarity. The fractions (46-55) by 60% and 80% methanol yielded about 75 mg of astragalin from *V.punctatum* and the fractions (36-46) by 90% and 99.8% methanol yielded about 35 mg of amentoflavone from *V.coriaceum*. The samples were also characterized by IR, NMR and Mass spectral studies for further confirmation, in addition to their melting point and homogeneity determination.

Astragalin and Amentoflavone[35]

Spectral Characterization

The spectral datas (IR, NMR and Mass) of the compound revealed that the compound to be astragalin ($C_{12}H_{20}O_{11}$, m.p. 178°C) as follows:

IR (KBr) ν cm^{-1} (KBr pellet of the sample)(Figure 2.): 3369.1 (-OH stretching); 3021.9 (aromatic–H stretching); 2997.3 (=C–H stretching); 2853.4–2925.5 (C–H stretching of glycoside moiety); 1732.1 (>C=O stretching); 1460.0 (C=C aromatic stretching) 1020.1-1215.9 (C–O stretching). ^{1}H-NMR (CD_3OD) 300 MHz, TMS, δ ppm: 6.68-7.19 (doublet, aromatic–C–H proton); 5.0 (singlet, aromatic–OH proton); 3.40-3.71 (multiplet, glycoside–C–H proton); 2.1 (multiplet, aliphatic–OH proton). ^{13}C-NMR (CD_3OD) 75 MHz, TMS, δ ppm: 178.5 (>C=O carbonyl carbon); 98.5-128.3 (aromatic carbon); 158.5-167.2 (aromatic substituted carbon); 135.4 (ethylene carbon); 62.2-93.4 (glycoside carbon). MS, m/z, [chemical fragments], (relative abundance in %): 448 $[C_{21}H_{20}O_{11}]^+$, molecular ion peak (19%); 449 $[C_{21}H_{20}O_{11}+H]^+$ (35%); 450 $[C_{21}H_{20}O_{17}+2H]^+$, (11%); 287 $[C_{15}H_{10}O_6+H]^+$, (100%); 288 $[C_{15}H_{10}O_6+2H]^+$, (20%); 289 $[C_{15}H_{10}O_6+3H]^+$, (05%).

Spectral Characterization

The molecular structure of the compound was further confirmed by spectral data of the isolated compound (amentoflavone, $C_{30}H_{18}O_{10}$, m.p. 300°C) as follows:

IR (KBr) ν cm^{-1}(Figure 3.): 3424.1 (-OH stretching); 3050-3000 (Aromatic-H stretching); 2997.3 (=C-H stretching); 1460 (C=C stretching Aromatic ring); 1726.8 (>C=O stretching); 1101.8 (C-O stretching). ^{1}H-NMR (C_6D_6) 300 MHz, TMS, δ ppm: 6.68-7.16 (multiplets, aromatic-H, ethylene proton); 5.0 (singlet, aromatic-OH proton). ^{13}C-NMR ($CDCl_3$) 75 MHz, TMS, δ ppm: 182.5 (carbonyl >C=O carbon); 160.2-166.5 (substituted aromatic carbon); 104.5 (ethylene carbon); 98.5-126.8 (un-substituted aromatic carbon). MS, m/z, [chemical fragments], (relative abundance %): 538.09 $[C_{30}H_{18}O_{10}]$ (32.4%); 539.09 $[C_{30}H_{18}O_{10}+H]^+$, (100%); 540.10 $[C_{30}H_{18}O_{10}+2H]^+$, (5.9%); 268.04 $[C_{15}H_9O_5-H]^+$, (1.5%).

Isolation of ferulic acid, arbutin and chlorogenic acid[36]

The genus *Viburnum* Linn. under the family-Caprifoliaceae (formerly) and Adoxaceae (recently) consists of about 200 species throughout the world. Many of these species have been reported to possess phenolic compounds and their glycosides, in addition to phyto-sterols and triterpenes, as their major chemical constituents in the stem and root barks. These chemical constituents may be attributed to their remarkable biological activities against smooth muscle related troubles and microbial infections (protozoal and bacterial origin). The main objective of the present study is to isolate and characterize some phenolic compounds from aqueous ethanolic stem extract of three *Viburnum* Linn. species, having antibacterial activity, collected from Nilgiri hills, Tamilnadu, India. The dried stems of *Viburnum punctatum* Buch.-Ham.ex D.Don, *Viburnum coriaceum* Blume and *Viburnum erubescens* Wall.ex DC. were powdered and extracted separately, successively with petroleum ether (60 – 80°C), chloroform, 75% aqueous ethanol. After the determination of percentage extractives, the residues were randomly tested against each of six strains of gram positive and gram negative bacteria at an approximate concentration (100 µg/ml) of all the test specimens using nutrient broth medium. The most sensitive strains were subjected to further investigations. The observation of the investigations revealed that the ethanolic stem residue of *V.erubescens* possessed a significant activity against *S.aureus*, *V.cholerae* and *E.coli,* when compared to the rest of the test samples. In particular, *V.erubescens* was far more potent against *P.aeruginosa* at all concentrations (25 – 800 µg/ml), when compared to the reference (Gentamycin, 25 – 800 µg/ml).

A preliminary organic analysis on the alcoholic stem extracts showed the presence of phenolic compounds such as flavonoids, tannins, anthocyanins, phenolic acids and their derivatives as their principal constituents, which may be attributed to a mild and pronounced antibacterial ativities of the extracts. In order to unfold the chemical constituents of the extract, the residues were fractionated in a column with solvents of increasing polarity followed by a gradient elution of components by methanol-water mixtures (decreasing polariity). About three phenolic compounds were isolated in pure form and spectroscopically characterized (UV, IR, NMR and Mass) to be ferulic acid, arbutin and chlorogenic acid from *V.punctatum*, *V.coriaceum* and *V.erubescens* respectively.

Phenolic glycoside and acids from *Viburnum* species

The substance was further subjected to IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopic analysis. The following are the spectral datas of the isolated compound [Ferulic acid ($C_{10}H_{10}O_4$, m.p.168 – 172°C)]:

IR (KBr) ν cm^{-1}: 3495.9 (–OH aromatic alcohol stretching); 3022.4 (aromatic–H stretching); 2984.5 (=C–H alkyl stretching); 1685.5 (>C=O carboxylic acid); 1521.7 (–C–C– aromatic ring); 1216.5 (–C–O– carboxylic acid, deformation); 1028.7 (–C–O methoxy group). ^{1}H-NMR (CD_3OD) 300 MHz, TMS, δ ppm: 11.213 (singlet, –COOH carboxyl proton); 7.762, 7.630 (doublet, ethylene gem proton); 6.653, 6.506, 6.312 (multiplet, Ar–H aromatic proton); 4.968 (–OH hydroxyl proton); 3.766 (singlet, –OCH_3 methoxy proton). ^{13}C-NMR ($CDCl_3$) 75 MHz, TMS, δ ppm: 167.85 (–COOH carboxyl carbon); 148.96, 147.80, 144.35 (Ar aromatic carbon); 125.68, 122.68 (aromatic carbon); 115.52 (ethylene carbon); 54.95 (–OCH_3 methyl carbon). MS, m/z, [chemical fragments], (relative abundance %): 194 [$C_{12}H_{16}O_7$] M^+, (0.5%) molecular ion peak; 177 [$C_{12}H_{16}O_7$–CH_3^+] (2%); 105 [$C_7H_5O^+$], (9.5%); 89 [$C_7H_5^+$], (100%); 77 [C_6H_5] $^{\bullet}$, (46%).

The isolated compound was subjected to spectral analysis to confirm the compound to be Arbutin ($C_{12}H_{16}O_7$, m.p. 145°C). The spectral data of the isolated compound as follows:

IR (KBr) ν cm^{-1}: 3429.5 (–OH stretching aromatic alcohol); 3022.3 (C–H aromatic-H stretching); 1522.0 (–C–C– aromatic ring); 1026.1 – 1216.6 (–C–O stretching). ^{1}H-NMR ($CDCl_3$) 300 MHz, TMS, δ ppm: 6.827, 6.624 (multiplets, Ar–H aromatic proton); 5.589 (singlet, carbon attached to –O – of glycoside); 5.101 (singlet, Ar–OH aromatic hydroxyl proton); 3.910, 3.79, 3.761 (multiplet, glycosidic carbon); 2950.2 (singlet, C–H alkane); 2.011 (singlet, –OH alcoholic hydroxyl proton). ^{13}C-NMR ($CDCl_3$) 75 MHz, TMS, δ ppm: 152.10, 151.27 (Aromatic carbon attached to –OH); 119.86, 117.09 (aromatic carbon); 102.26 (glycosidic carbon attached to O–side chain); 76.89, 76.4, 73.8, 70.2 (glycosidic carbon); 61.38 (glycosidic

side chain carbon attached to –OH). MS, m/z, [chemical fragments], (relative abundance %): 272 [$C_{12}H_{16}O_7$, M^+], (100%) molecular ion peak or base peak; 273 [$C_{12}H_{16}O_7$+H], (13.2%); 274 [M+2], (1.4%); 93 [$C_6H_5O_7^+$]•, (6.6%) ; 179 [$C_6H_{11}O_6^+$]•, (6.8%).

Chlorogenic acid

IR (KBr) ν cm^{-1}: 3395.8 (–OH aromatic alcohol stretching); 3024.6 (Ar–H aromatic ring stretching); 2960.1, (CH=CH stretching); 1217.9, (C–O stretching); 1650.7, (>C=O stretching); 1554.1, (–C=C– aromatic ring). ^{1}H-NMR (CD_3OD) 300 MHz, TMS, δ ppm: 10.998 (singlet, –COOH carboxyl proton); 7.652 (doublet, ethylene CH=CH); 6.689, 6.657, 6.501 (multiplet, Ar–H); 5.101 (doublet, Ar–OH); 3.968, 3.843, 3.298, 3.202 (multiplet, cyclohexane proton); 2.189 (singlet, Alcoholic–OH); 1.95, 1.85 (doublet, $–CH_2$ proton of cyclohexane). ^{13}C-NMR (CD_3OD) 75 MHz, TMS, δ ppm: 77.45 (–COOH carboxylic carbon); 166.51 (carboxyl carbon); 147.01, 145.92, 144.76 (Aromatic carbon); 122.52, 121.23 (Ethylene carbon); 76.33, 67.01, 62.96 [Substituted aliphatic ring carbon (cyclohexane)]; 38.73, 35.84 (Un-substituted aliphatic ring carbon). MS, m/z, [chemical fragments], (relative abundance %): 354 [$C_{16}H_{18}O_9$, M^+], (2.5%) molecular ion peak; 355 [$C_{16}H_{18}O_9$+H], (59%); 356 [$C_{16}H_{18}O_9$+2H^+], (1%); 163 [$C_9H_7O_3$], (100%) parent ion peak; 135 [$C_8H_7O_2$], (7%).

Antibacterial potential of *Viburnum* species

A preliminary organic analysis on the alcoholic stem extracts showed the presence of phenolic compounds such as flavonoids, tannins, anthocyanins, phenolic acids and their derivatives (glycosides) as their principal constituents, which may be attributed to a mild and pronounced antibacterial ativities of the extracts. In order to unfold the chemistry of constituents of the extracts, the residues were fractionated in a glass column with solvents of increasing polarity followed by a gradient elution of components by methanol-water mixtures (decreasing polariity). About three phenolic compounds were isolated in pure form and spectroscopically characterized (UV, IR, NMR and Mass) to be ferulic acid, arbutin and chlorogenic acid from *V.punctatum*, *V.coriaceum* and *V.erubescens* respectively. It has been finally concluded that a part of the antibacterial spectrum may be provided by the isolated compounds.

Antibacterial activity of ethanolic stem extracts

The following factors were employed for susceptibility of microbes: Solvent Used- Dimethyl sulfoxide; Standard Antibiotic used- Gentamycin; Concentrations screened- 25, 50, 100, 200, 400, 800 μg; Sample preparation-10 mg sample was dissolved in 1 ml of DMSO; Stock Sample Concentration- 10 mg/ml; Method-Agar diffusion method; Bacteria analyzed- *Staphylococcus aureus, Vibrio cholerae, Escherichia coli, Pseudomonas aeruginosa*; Media Used-Peptone-10 g, NaCl-10g and Yeast extract 5g, Agar 20g in 1000 ml of distilled water

Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 h in an incubator. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 μl, 10^{-4} cfu) and spread evenly on the plates. After 20 min, the wells were filled with of test compounds (25, 50, 100, 200, 400 and 800 μg). The control wells for Gentamycin were also prepared same as above described. All the plates were incubated at 37°C for 24 h and the diameter of inhibition zone were noted. The results were then tabulated in terms of the concentration dependant Zone of Inhibition (ZOI) and Minimum Inhibitory Concentration (MIC).

Isolation of astragalin and amentoflavone from the roots of *Viburnum erubescens* Wall.ex DC and their effect against human pathogenic bacteria[37]

The hydroalcoholic extract gave the highest yield when compared to both water and other moderately and highly polar solvents and the colour formation upon qualitative chemical analysis on the hydroalcoholic fraction was observed to be highly pronounced in addition to a positive test for phenolics, flavonoids, saponins, carbohydrates, amino acids and glycosides (the glycosides were determined to be phenolic in nature after exhausting free sugar followed by hydrolytic test. The colour of the fluorescence analysis was markable to the polar extracts. So, it was decided to select 75% hydroalcoholic extract for further phytochemical investigation such as isolation of phenolic

compounds with appropriate solvents using a column chromatography.

Spectral Characterization

The spectral datas (^{13}NMR and Mass) of the compound revealed that the compound to be astragalin ($C_{12}H_{20}O_{11,}$ m.p. 178°C) as follows:

^{13}C-NMR (CD_3OD) 75 MHz, TMS, δ ppm: 178.5 (>C=O carbonyl carbon); 98.5-128.3 (aromatic carbon); 158.5-167.2 (aromatic substituted carbon); 135.4 (ethylene carbon); 62.2-93.4 (glycoside carbon) (Figure 1). MS, m/z, [chemical fragments], (relative abundance in %): 448 $[C_{21}H_{20}O_{11}]^+$, molecular ion peak (19%); 449 $[C_{21}H_{20}O_{11}+H]^+$ (35%); 450 $[C_{21}H_{20}O_{17}+2H]^+$, (11%); 287 $[C_{15}H_{10}O_6+H]^+$, (100%); 288 $[C_{15}H_{10}O_6+2H]^+$, (20%); 289 $[C_{15}H_{10}O_6+3H]^+$, (05%).

Spectral Characterization

The molecular structure of the compound was further confirmed by spectral data of the isolated compound (amentoflavone, $C_{30}H_{18}O_{10}$, m.p. 300°C) as follows:

^{13}C-NMR ($CDCl_3$) 75 MHz, TMS, δ ppm: 182.5 (carbonyl >C=O carbon); 160.2-166.5 (substituted aromatic carbon); 104.5 (ethylene carbon); 98.5-126.8 (un-substituted aromatic carbon) (Figure 3). MS, m/z, [chemical fragments], (relative abundance %): 538.09 $[C_{30}H_{18}O_{10}]$ (32.4%); 539.09 $[C_{30}H_{18}O_{10}+H]^+$, (100%); 540.10 $[C_{30}H_{18}O_{10}+2H]^+$, (5.9%); 268.04 $[C_{15}H_9O_5\text{-}H]^+$, (1.5%)

For the current study about four strains of bacteria were obtained (Biogenics, Karnataka, India) to test their susceptibility (concentration dependent) in terms of Minimum Inhibitory Concentration (MIC) and Zone of Inhibition (ZOI) against a standard antibiotic. In this event, a mixture of astragalin and amentoflavone showed an MIC 200 µg to a ZOI 1.2 cm against the standard Gentamycin, whose MIC being 25 µg and ZOI, 3.1 cm as far as *S.aureus* in concerned.Against *V.cholerae*, the test drug showed its MIC at 50 µg with a ZOI of 0.7 cm at 800 µg concentration whereas the standard, however, showed one half of the dose when compared to the test. Against *E.coli*, the test was as equally effective as that of standard drug i.e, 25 µg as MIC however, ZOI of the standard the later was however dominant. As fas as the last strain namely *P.aeruginosa* was concerned, the test sample showed its MIC as that of the standard subjected i.e, 25 µg at a concentration of 800 µg level with a ZOI vice, 1.4 and 1.8 cm respectively. Hence it can be stated that at a concentration of 800 µg level, a mixture of astragalin and amentoflavone which have been isolated from *V.erubescens* root is showing activity either comparable or equal as that of the standard subjected (Gentamycin at a dose level of 800 µg) *in vitro*.

A study on physico-chemical standardization of a formulated Triple *Viburnum* root Asava possessing Anti-helmintic activity[38]

Physico-chemical parameters

The term total solid is applied to the residue obtained where the prescribed amount of the preparation is dried to constant weight. The total solid of the asava were determined to be 51.6±0.2% w/ml. The lower limit of the range is the temperature indicated by the thermometer when the first drop of condensate leaves the tip of the condenser and the upper limit is the temperature at which the last drop evaporates from the lowest point in the distillation flask, as far as distilling range of the asava is concerned. In this event, the asava showed 72±0.02° C to 110±0.08° C as its boiling range. The congealing temperature is that point at which there exists a mixture of the liquid (fused) phase of a substance and increasing proportion of solid phase. The asava, in this case, showed 62±0.08° C to 65±0.04° C as the congealing point. Making no modification in the setting of apparatus the freezing point of the asava was determined to be 10±0.06° C. Since the principle behind the formulation of asava is that conversion of sugar (jaggery) in to ethanol by anaerobic fermentation process, the total alcohol concentration was determined to be 21% v/v at 32° C by distillation cum specific gravity method. Loss on drying is a versatile method of standardization applicable for materials existing in liquid, solid, semisolid state. On the basis of the above principle, loss on drying of the asava was determined to be 33.42±0.60% w/w. Although loss on ignition is best suiting to standardize formulation such as churna, it cannot be stated that arista may not be standardizable by this method. Because, the principle behind the loss on ignition is to determine the quantity of inorganic elements which could be convertible in to

their corresponding oxides, which include both physiological as well as non-physiological ashes. Hence, the loss on ignition of the asava in percentage w/v as determined to be 3.9±0.33% w/v. To determine the acidity or alkalinity of the asava, pH value was determined to be 4.6 by potentiometric method. Determination of refractive index is one of the best suiting standardizing process for liquid formulation with reference to air; the refractive index of the asava using as Abbe refractometer against water was measured to be 1.299. By employing an Oswald - type viscometer, viscosity was determined against water to be 1.9994 poise at 32°C. Since asava is a liquid formulation, by using a calibrated Pycnometer, the weight per ml of the asava was determined to be 1.135 g/ml at room temperature. The total free sugar content using Benedict's reagent for quantitative analysis was determined to be 25 g %.

Anthelmintic potential

The earthworms introduced to distilled water (group-I, control) were very alive and showed neither signs of paralysis nor any death. The group-II, which was treated with reference anthelmintic drug (piperazine citrate 10 mg/ml, showed a significant paralysis of worms, 21.50±1.02), however, no death was evident.The group III, IV and V, which were introduced to the solution containing the arista equivalent to 100, 250 and 500 mg/ml respectively, resulted both paralysis (35.81±0.49, 22.54±0.55 and 15.41±0.37) as well as death (95.01±2.33, 65.47±0.94 and 31.11±0.42) significantly. Hence, the paralytic capacity of the arista was comparable to that of the reference subjected, but the same time, the formulation is more powerful and effective than the standard in causing a significant morality of the earth worms $p<0.01$. The phenolic compounds such As tannins and flavonoids bind with intestinal enzymes of earthworms as well as the glycoprotein part of the cuticular layer of the worms thereby, crippling the protein dependent movements of the body (muscles) of the worms leading to an irreversible paralysis and finally to death.

Isolation of chlorogenic acid from stems *Viburnum coriaceum*[39]

The dried stems of *Viburnum coriaceum* Blume. were powdered and extracted separately and successively with petroleum ether (60 – 80°C), chloroform and 75% aqueous ethanol. The ethanolic extracts were screened by Thin Layer Chromatographic techniques for the presence of different classes of phenolic compounds. The TLC studies revealed the presence of some flavonoid which was later confirmed to be chlorogenic acid in V.coriaceum by co-TLC technique against the reference substances. The ethanolic residues were adsorbed on the activated neutral alumina and packed in a glass column containing slurry of alumina in acetone. Then, the column was eluted firstly with some non-polar and moderately polar solvents to clear out impurities which remain present in the column followed by a gradient elution with methanol-water mixture. The fractions yielded 35 mg of isolated compound.

A small quantity of the substance was dissolved in ethyl acetate and run on a TLC using absolute ethanol as developing phase and silicagel-G as stationary phase. The chromatogram showed a distinct spot against the spray of 3% H2SO4 (methanolic) Rf-0.46 (a dark green spot). The crude sample was crystallized repeatedly from distilled water and stored at 4°C to get about 95 mg of crystals. The spectral studies resulted the following: IR (KBr) ν cm-1: 3395.8 (–OH aromatic alcohol stretching); 3024.6 (Ar–H aromatic ring stretching); 2960.1, (CH=CH stretching); 1217.9, (C–O stretching); 1650.7, (>C=O stretching); 1554.1, (–C=C– aromatic ring). 1H-NMR (CD3OD) 300 MHz, TMS, δ ppm: 10.998 (singlet, –COOH carboxyl proton); 7.652 (doublet, ethylene CH=CH); 6.689, 6.657, 6.501 (multiplet, Ar–H); 5.101 (doublet, Ar–OH); 3.968, 3.843, 3.298, 3.202 (multiplet, cyclohexane proton); 2.189 (singlet, Alcoholic–OH); 1.95, 1.85 (doublet, –CH2 proton of cyclohexane).

Isolation of arbutin from roots of *Viburnum erubescens*[40]

Following a successive extraction and a primary organic analysis, it has been known to contain phenolic compounds as its principal phyto-constituents in their hydroalcoholic fraction. From 1.25 kg of the root extract, approximately 40 mg of the isolated compound was subjected to spectral analysis to confirm the compound to be Arbutin ($C_{12}H_{16}O_7$, m.p. 145°C). The spectral data of the isolated compound as follows: IR (KBr) ν cm^{-1}: 3429.5 (–OH stretching aromatic

alcohol); 3022.3 (C–H aromatic-H stretching); 1522.0 (–C–C– aromatic ring); 1026.1 – 1216.6 (–C–O stretching) (Figure 1). ^{1}H-NMR ($CDCl_3$) 300 MHz, TMS, δ ppm: 6.827, 6.624 (multiplets, Ar–H aromatic proton); 5.589 (singlet, carbon attached to –O – of glycoside); 5.101 (singlet, Ar–OH aromatic hydroxyl proton); 3.910, 3.79, 3.761 (multiplet, glycosidic carbon); 2950.2 (singlet, C–H alkane); 2.011 (singlet, –OH alcoholic hydroxyl proton).

Figure 1: Literature review on *Viburnum Linn.* species

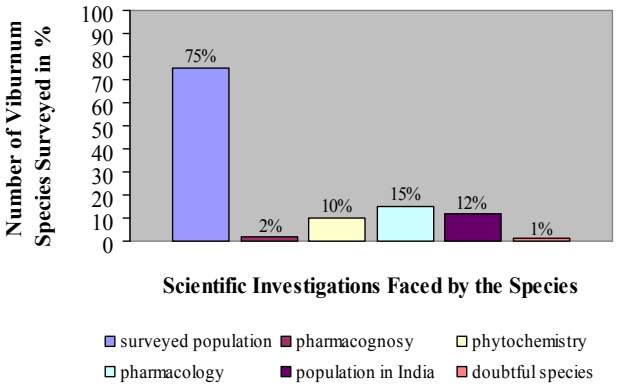

Figure 2: IR spectra of astragalin isolated from stem ethanolic extract of *V.punctatum*

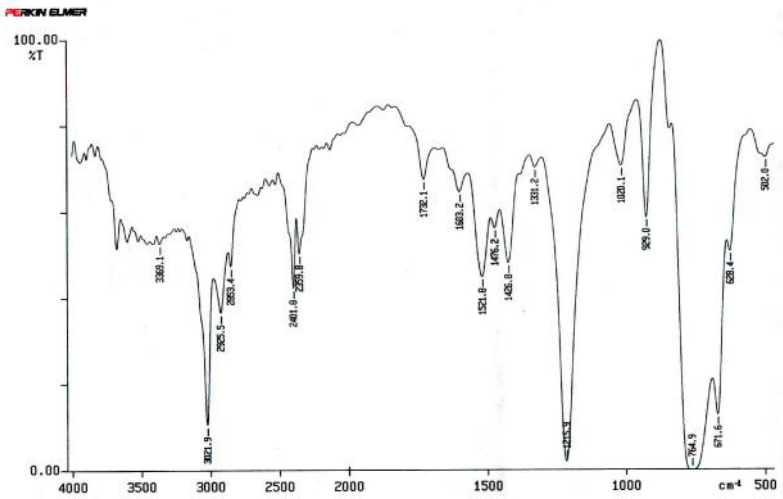

Figure 3: IR spectra of amentoflavone isolated from stem ethanolic extract of *V.coriaceum*

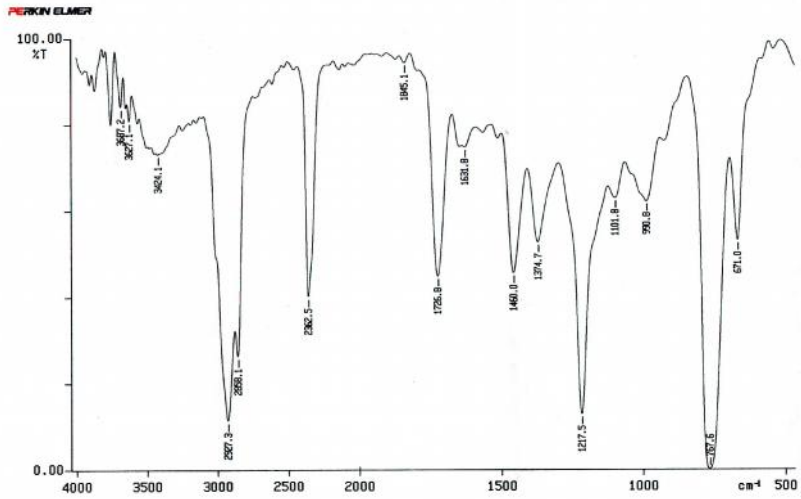

Table 1: Standardization of arista by physical and physic-chemical methods

S.No.	Parameters	Report/Values
1.	Total solids	46.5±0.15% w/ml
2.	Boiling range	70±0.04 – 105±0.06° C
3.	Congealing point	62±0.008 – 65±0.06° C
4.	Content of ethanol	21% v/v at 32° C
5.	Freezing point	8±0.08° C
6.	Loss on drying	18.12±0.50% w/w
7.	Loss on Ignition	3.1±0.33% w/v
8.	pH	4.5
9.	Refractive Index against water (1.332)	1.440
10.	Viscosity against water (0.9982)	1.9096 poise at 32° C
11.	Weight per ml	1.036 g/ml
12.	Total free sugar content	20 g % w/ml
13.	Fluorescence analysis (Long UV)	
	a. Arista	Dark brown
	b. Arista in water	Yellowish brown
	c. Arista with methanol	Yellowish brown
	d. Arista with ethylacetate	Pale brown

Results are presented as mean ± Standard Deviation, n=3

Table 2: Effect of *Viburnum* churna and Indomethacin on cotton pellet-induced granulom formation in rats.

Group	Treatment	Wet weight (mg)	Dry weight (mg)	% Inhibition
I	1% CMC	178.13 ± 7.45	63. 40 ± 2.88	-
II	Triple *Viburnum* root Churna 500 mg/kg	152.47 ± 5.64*	31.18 ± 4.53*	50.17 %
III	Indomethacin 20 mg/kg	133. 87 ± 8.20*	17.24 ± 1.89*	73.28 %

Values are presented as mean ± SEM from 6 animals in each group. *p < 0.001

Table3: Efect of *Viburnum* leaf arista on biochemical parameters in water immersion plus restraint stress induced ulcer

Treatment Groups	Severity index of bleeding	Ulcer score	Superoxide dismutase (unit/mg protein)	Catalase (µ moles of H_2O_2 consumed/min/mg protein)	Lipid peroxidation (n moles of MDA/mg protein)	Gastric wall mucus content (Absorbance at 598 nm/g wet tissue)
Normal control	0.0±0.0	0.0±0.0	66.10 ± 3. 24	81.11 ± 2.81	111.31 ± 6.44	1.78 ± 0.12
1% SCMC 10 ml/kg b.w	2.94 ± 0.18*	31.83 ± 2.11*	201.50 ± 8.43*	28.40 ± 1.86*	198.94 ± 11.15*	0.41± 0.01*
300 mg arista equivalent/kg b.w	0.63 ± 0.41*	16.99±1. 32*	112.44 ± 5.04*	39.14 ± 3.01#	58.22 ± 4.20*	0.65± 0.20#

Values are presented as mean ± SEM of 6 animals.

Symbols represent statistical significance: #- p< 0.05, * - p< 0.001

Table 4: Anti-inflammatory activity of *Viburnum* churna on carrageenan induced paw oedema

Treatment Groups	1st hour	2nd hour	3rd hour	4th hour	5th hour
Control	0.17±0.010	0.26±0.035NS	0.32±0.041NS	0.34±0.068NS	0.37±0.081NS
Triple *Viburnum* Churna	0.18±0.014 (5.88)	0.24±0.017* (7.69)	0.20±0.011** (37.5)	0.18±0.016** (47.05)	0.12±0.009** (67.56)
Indomethacin 10 mg/kg	0.17±0.014 (0)	0.22±0.001* (15.38)	0.19±0.010** (40.62)	0.15±0.014** (55.88)	0.11±0.012** (70.27)

Values are presented as mean ± SEM from 6 animals in each group and values in parenthesis percentage inhibition.

Symbols represent statistical significance: *– p<0.01, **–p<0.001, NS – non significant, Unit: ml

Table 5: Effect of *Viburnum* asava on Castor oil induced diarrhoea

S.No.	Treatment/Dose (mg/kg)	Mean weight of defecation after 4 h	Percentage inhibition of defecation
1.	Control (Tween-80, 2 ml/kg)	22.09±0.47	-
2.	*Viburnum* asava (200 mg/kg)	13.68±0.18	38.17±1.64*
3.	*Viburnum* asava (400 mg/kg)	10.25±0.31	53.49±2.17**
4.	Loperamide (1 mg/kg)	6.11±0.26	72.63±1.42**

Values are presented as mean ± SEM of 6 animals. Symbols represent statistical significance: * - $p < 0.01$, ** - $p < 0.001$

Table 6: Histo-chemical analysis on the leaves, stem and roots of three *Viburnum* Linn. species

Plant species	Plant organ	Histological region	Phyto-constituents present
V.punctatum	Leaf	Phloem region	Tannins
		Mesophyll	Mucilage
		Vascular region	Lingin
	Stem	Xylem ray cells	Tannins
		Phloem parenchyma	Starch grains
		Xylem region	Lignin
		Pith cells	Saponins
	Root	Xylem ray cells	Tannins
		Xylem parenchyma	Starch grains
		Xylem region	Lignin & saponins
V.coriaceum	Leaf	Mesophyll	Tannins
		Ground tissue	Druses
		Xylem parenchyma	Saponins & lignin
	Stem	Xylem parenchyma	Lignin
		Phloem parenchyma	Starch grains
		Xylem ray cells	Tannins
		Cortex	Druses
		Pith cells	Saponins
	Root	Xylem vessel	Lignin
		Xylem parenchyma	Starch grains
		Xylem region	Saponins
		Xylem ray cells	Tannins
V.erubescens	Leaf	Phloem region	Tannins
		Trichomes	Lignin
		Vascular bundle	Lignin
		Phloem parenchyma	Saponins
	Stem	Ground tissue	Starch grains (rarely)
		Phloem ray cells	Tannins
		Phloem region	Starch grains
		Xylem ray cells	Starch grains
		Periderm	Suberin
	Root	Phloem region	Saponin
		Xylem	Lignin
		Phloem ray cells	Tanins
		Xylem	Lignin
		Cortical parenchyma	Starch grains
		Phloem region	Sapoins
		Periderm	Suberin

Conclusion

In spite of its big population, the numbers of *Viburnum* species which have been scientifically investigated are very few. This article centres on some recent biological and phytochemical analysis carried out on the three of the *viburnum* Linn.species. We also hope this article will definitely make awareness among pharmacognosist and other researchers to progress further scientific investigations on these species as well as their co-species.

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