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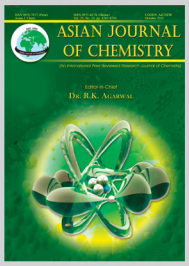

Formulation and Physico-Chemical Standardization of Triple *Viburnum* Leaf Arista and its Antiulcer Potentials

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A Triple *Viburnum* arista by anaerobic fermentation process was prepared. The formulated arista was standardized by some physico-chemical methods to obtain consistent parameters. The ED_{50} value of arista was calculated by Committee for the Purpose of Control and Supervision of experiments on animals (CPCSEA) guidelines method in terms of mg of arista per kg body weight equivalent to evaluate arista for its antiulcer potential in wistar rats. Water immersion plus restraint stress induced ulcer model was selected to study or screen the antiulcer potentials of the formulation against its solvent control group. The arista showed a significant antiulcer effect when the status of biochemical parameters such as superoxide dismutase; catalase; lipid peroxidation; gastric wall mucus were estimated and compared with the gastric juice of a healthy animal. The results observed were statistically significant ($p < 0.001$).

Key Words: *Viburnum*, Arista, Patha, Physico-chemical, Antiulcer.

INTRODUCTION

The genus *Viburnum* Linn. species under the family Caprifoliaceae (formerly) and Adoxaceae (recently) includes about 200 species distributed throughout the world and about 17 of them have been reported in India. Their growth is favoured at an altitude from 1500-2500 ft and are frequently seen in Himalayan tracts, Nilgiri hills and Coimbatore[1,2].

Viburnum Linn. species have been reported to contain sesquiterpenes[3], triterpenes and phytosterols; phenolic compounds and their glycosides such as tannins, flavonoids and anthocyanins, irridoid glycosides on their stem, root and leaves and investigated to posses uterine sedative, diuretic, cardiovascular stimulant, antimicrobial, antiinflammatory, antinociceptive, antispasmodic, antiasthmatic and astringent activities[4]. In the late 1960s and early 1980s, the magnitude of scientific investigations on the genus *Viburnum* Linn. were voluminous in regard to some phytochemical aspects of constituents from the stems, root barks and leaves of these species[5-7]. However, the number of species exploited for studies and areas of investigations were very limited. After a couple of decades, some more *Viburnum* species appeared for having been investigated of their phytochemical and pharmacological characteristics. The typical examples are: iridoid aldehydes and their glycosides in *Viburnum luzonicum*[8] and their cytotoxic effect; vibsane type diterpene from *Viburnum awabuki*[9]; irdoid glycosides from *Viburnum tinos*; antinociceptive and antiinflammatory activities of *Viburnum lanata*[10] and *Viburnum opulus*[11] and an iridoid glucoside from *Viburnum rhytidophyllum*[12]. A detailed pharmacognostical studies have, recently, been carried out on a few of the species, since the same species have been screened for their antibacterial spectrum also[13,14].

In addition to the above, a questionnaire and a verbal enquiry have been recently conducted to the local dwellers, tribal and the herbalists of Nilgiri hills and Coimbatore hills, Tamilnadu, India, about the ethno-pharmacological status of some *Viburnum* species, has also revealed that the leaves, stem bark and root barks of mature plants had been reliably in usage to the non-pregnant uterus[15], the GIT related ailments and are also in application as an ideal healing aid against inflammation[16]. Accounting the above information, it was decided to formulate an arista from three *Viburnum* leaves and the same was screened for antiulcer effect after its standardization employing some physico-chemical methods.

EXPERIMENTAL

The leaves of *V. punctatum*, *V. coriaceum* and *V. erubescens* were collected (flowering season, June-August)

from Nilgiri hills, Tamilnadu, India and authentificated by Dr. V. Chelladurai, Ex. Professor, (Botany), Medicinal plant survey for Siddha, Government of India, as *Viburnum punctatum* Buch.-Ham.ex D.Don (VP), *Viburnum coriaceum* Blume (VC) and *Viburnum erubescens* Wall.ex DC (VE). Herbarium of the specimens (labeled V181, VC131 and VE131 for VP, VC and VE respectively) was submitted to the museum of the Department of Pharmacognosy, Nandini Nagar Mahavidyalaya College of Pharmacy.

Preparation of Triple *Viburnum* leaf arista by anaerobic fermentation method (An ayurvedic formulation): About 1.5 seers (60 g) of the leaves (each 20 g, 1:1:1 ratio)(patha) were coarsely powdered and added with 32 seers (1024 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[17]. The decoction was taken in wooden vats of 2 L capacity, to which dissolved were 121/2 seers (400 g) of jaggery and boiled for 0.5 h.

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)[18,19].

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 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 and biological screening.

Standardization of arista

Determination of total solids: A shallow, flat bottomed flanged dish, about 75 mm in diameter and about 25 mm deep, made of nickel was used for this analysis. Accurately 5 mL of arista was pipetted out and placed in the dish and evaporated at low temperature on a water bath until the solvent was removed and the residue is apparently dry. Then the dish was placed in an oven and dried to constant weight at 105 ºC. After the dish was provided with well-fitting cover, it was cooled in a desiccators[20].

Determination of boiling range (distilling range): A distillation unit fit with a thermometer was employed to determine the boiling range of the arista. The apparatus consisted of a distilling flask of 200 mL capacity, a condenser of 60 cm long, a receiver of 100 mL capacity which was graduated with 1 mL division and a thermometer showing 0-240 ºC.

The thermometer was positioned in the centre of the neck and the entire assembly was shield after dropping about 100 mL of arista to the distilling flask. With the aid of metallic stand and clamps, the entire assembly was placed on an electric heater having a thermostat, so that adjustment in temperature could be done conveniently. Distillation was switched on and the recorded was the temperature of first drop of the distillate. Then the temperature was increased in such a way the receiver could collect 4-5 mL per min. The process was continued until 25 % (25 mL) of the distillate reached the receiver and the temperature of the last drop of the distillate to the receiver was also noted.

Necessary correction was employed observing the temperature readings from any variation in the parametric pressure from the normal (101.3 kPa) using following expression.

$$t_1 = t_2 + K(a - b)$$

t_1 = corrected temperature; t_2 = the observed temperature; a = 101.3; b = the barometric pressure of the time of the determination; K = the correction factor[20].

Determination of congealing range or temperature: The congealing temperature is that point at which there exists a mixture of the liquid phase of a substance and a larger proportion of the solid phase. This experimentation required 1 L beaker in which two test tubes were placed in such a way one was inserted in to another test tube. The inner test tube contained 15 mL of arista and stopperd with a cork attached with a stirrer and a thermometer with 0.2 ºC graduation.

The beaker was filled with water and the test tubes were clamped in such a way they were immersed in water and distance of 18 mm be maintained between the bottoms of the beaker and test tube. The temperature at which a substance solidifies upon cooling is a useful index of purity[20].

Preparation of reference substance: Since arista is a liquid, the process of determination of congealing point was carried out in the same way of raising temperature, while stirring, about the room temperature using the apparatus for congealing point determination and noted down as a reference value.

Preparation of test substance of arista: The temperature of the bath was maintained near 15 ºC using addition of ice cubes and placed on a heating mantle which was kept turned off. Then the sample was stirred constantly to a rate of 20 cycles per min with simultaneous observation of rise in temperature with the thermometer. The congealing point was still hidden up to the room temperature. Hence, a slow rise of temperature was aided to the bath using the heating mantle until the congealing point appeared which was comparable to that of the standard. The process was repeated three times and the average was tabulated.

Determination of ethanol: 25 mL of arista were accurately measured and mixed with 100 mL of double distilled water and poured in to a separating funnel. The mixture was saturated with sodium chloride and add 100 mL of hexane, shaken vigorously 2-3 min. The mixture was allowed to stand for 0.5 h. The lower layer was run in to a distillation flask. The hexane layer was washed with 25 mL of concentrated sodium chloride solution in a separating funnel then the NaCl layer was added to the distillation flask. The whole mixture was made alkaline with 1 M sodium hydroxide solution using solid phenolphthalein as indicator. To this add a little pumice powder and 100 mL of water.

The whole mixture was distilled to obtain 90 mL of distillate. The distillate was poured in to a 100 mL volumetric flask and made the volume to 100 mL with double distilled water. Using this mixture relative density was determined to calculate the percentage v/v alcohol of the arista[20].

Determination of freezing point of arista: Freezing point is the maximum temperature occurring during the solidification of a super-cooled liquid. The apparatus for its determination was designed as that of the apparatus used in the determination of congealing point of arista.

About 5 mL of arista was placed in the inner test tube, which was immersed in a 500 mL capacity beaker containing water, fitted with a thermometer and a stirrer. The stirring was carried out at a rate of 25 cycles per min with simultaneous reduction in temperature by keep on adding ice cubes. When the temperature of the arista was observed to be 5 ºC or below, the beaker was filled with saturated NaCl solution to stabilize or maintain temperature. The process was continued until some seed crystals of arista were present. The process was repeated 3 times at least to get the average freezing point of arista[20].

Loss on drying: About 10 mL (11.02 g) of the arista under study were accurately pipetted out and transferred to a tarred china dish which was known for its weight and kept in a hot air oven at 100-105 ºC for 1 h. Then, the sample was weighed along with china dish to deduct the actual weight of tarred china dish. The weight of the content was noted to calculate the percentage loss on drying with reference to the arista[20].

Determination of loss of ignition: Though determination of loss on ignition is best suiting solid formulation like churna and the principle behind it is to convert all metallic oxalate, chloride, sulphate, phosphate, silicate *etc*., in to their concerned oxide form.

Arista is a liquid formulation containing active principle in alcohol along with minerals in its aqueous layer or unfiltered fine crude drug particle during the preparatory moments. Hence, this method of standardization was tried with 10 mL arista also using a silica crucible, after allowing arista be auto-evaporated at room temperature[20] for *ca.* 1 h.

Loss on ignition: A silica crucible was heated for *ca.* 0.5 h to red hot and cooled in a desiccator to note down its weight. About 10 mL of the arista was pipette out and then dried at 100-105 ºC for 1 h and ignited to constant weight in a muffle furnace at 600-625 ºC, until a carbon free ash formed. The crucible was allowed to cool in a desiccator after each ignition and care was taken to avoid catching fire. The weight of the carbon free ash was determined. The procedure was repeated to obtain a standard deviation to ensure consistency and then tabulated.

Determination of pH of arista: To determine the acidity or alkalinity of the arista at room temperature, potentiometric method was employed. The buffer solutions A-H were prepared using carbon dioxide free water as solvent as given in Indian Pharmacopoeia-1996 (A-95) which helped to detect the pH of arista whose range[21] may be from 1.7-10.12.

Determination of refractive index: The refractive index (n) of a substance with reference to air is the ratio of the sine of angle of incidence to the sine of the angle of refraction of beam of passing from air in to the substance. The refractive index was conveniently measured using the Abbe refractometer at 25 ºC employing the wavelength of the D line of sodium ($\lambda = 589.3$ nm), after calibrating the apparatus against distilled water[21] whose nD^{20} at 25 ºC was 1.3225.

Determination of viscosity of arista: The determination of viscosity of arista was carried out by means of capillary viscometer at room temperature. The viscometer was washed and dried completely. Then the viscometer was filled and examined through L tube to slightly above the mark G using a long pipette to minimize wetting the tube above the mark. The tube was placed vertically in a water bath maintained at 35 ºC and allowed to stand for 0.5 h to reach equilibrium. The volume of arista was adjusted so that the bottom of the meniscus settled at the mark G. The liquid was sucked to the point about 5 mm above the mark E and the pressure was revealed[21].

The time taken was measured for the bottom of the meniscus to fall from the top of mark E to the top edge of mark F. Then, the kinematic viscosity (V) in square mm per sec ($mm^2\ s^{-1}$) using the expression

$$V = Kt$$

The constant (K) of the instrument was determined on a liquid of known viscosity (Dextran injection or saline).

Determination of weight per mL of arista: The weight per mL of a liquid is the weight, in g, of 1 mL of the liquid when weighed in air at room temperature. A thoroughly clean and dry Pycnometer was selected and filled with arista and weighed in air at room temperature. The procedure was repeated 3 times and average value of the weight of 1 mL of arista was calculated[21].

Primary organic analysis: About 100 g of the crude drug (Patha) were powdered in a mechanical grinder, after a screening for the presence of foreign bodies, in to a moderately coarse powder were soxhleted successively with solvents of increasing polarity such as petroleum ether, benzene, chloroform and 75 % ethanol (15-19 h) and a part of the extracts and the arista were subjected for the determination of and a primary organic analysis.

Primary organic analysis of the both the extracts and the arista were carried out with suitable chemical reagents of research grade which led to a conclusion that the phenolic compounds were well pronounced[22].

Determination of total free sugar content in arista: The total free sugar content of arista was estimated using Benedict's reagent for quantitative analysis and reported in terms of percentage w/mL as per the reference[23].

Antiulcer screening: Animal studies were approved by Intuitional animal Ethics Committee (IAEC) of DOABA college of pharmacy, Mohali, Punjab, India and carried out in accordance with committee for the pupose of control and supervision of experiments on animals (CPCSEA) guidelines.

The statistical analysis of the study was carried out using one way analysis of variance (ANOVA) followed Dunnett's 't' test and all calculations were performed using Graph-pad Prism software, $p < 0.05$ was accounted significant.

By acute oral toxicity method LD_{50} of the arista in animals were determined followed by determination ED_{50} that was used in the study.

Water immersion plus restraint stress-induced gastric ulcer (WIRS): Wistar albino rats of either sex weighing 200-250 g were selected. Rats were fed with standard chow diet and water ad libitum till the end of the experimental period. Distributions of the animals in-group, sequence of trials and treatment aspects were randomized.

Animals were divided into three groups of six animals each. Animals were placed in cages with grating floor to avoid coprophagy and fasted for 12 h following free access to water.

Group-I-normal control. Group-II-received 1 % SCMC 10mL/Kg b.w. (p.o.). Group-III-received 300 mg arista equivalent/kg b.w. (p.o.) suspended in 1 % SCMC.

The experimental rats were immobilized under light ether anaesthesia by strapping the rats on a wooden plank[24] and subsequently they were immersed in water up to xiphoid process for 7 h. The temperature of the water was maintained at 24 ± 1 ºC. Drugs were given orally 0.5 h prior to the restraint procedure. After 7 h of immobilization and water immersion the animals were taken out and sacrificed with high-dose anesthetic ether. The stomach was removed and the severity of intra-luminal bleeding was examined and expressed as score for intensity (SI) of intra-luminal bleeding according to the following scale, 0 = no blood detectable; 1 = thin blood follows the rugae; 2 = thick blood follows the rugae; 3 = thick blood follows the rugae with blood clots in certain areas; 4 = extensive covering of the whole of mucosal surface with thick blood.

After wiping the blood, the ulcer score was determined and the stomach tissue was subjected to gastric wall mucus content, lipid peroxidation, superoxide dismutase and catalase.

Status of biochemical parameters

Estimation of superoxide dismutase (SOD): Superoxide dismutase was estimated using 0.1M carbonate bicarbonate buffer, pH 10.2, containing 5.7 mg EDTA/100 mL and 3 mM epinephrine.

To 0.05 mL of stomach homogenate, 1.5 mL of the buffer was added. The reaction was initiated by the addition of 0.4 mL epinephrine and change in the optical density per min was measured at 480 nm. One unit of superoxide dismutase activity is the amount of enzyme required to give 50 % inhibition of epinephrine auto-oxidation[25].

Estimation of catalase (CAT): Catalase was estimated according to the following method: 50 mM phosphate buffer, pH 7.0 and 30 mM Hydrogen peroxide in phosphate buffer.

To 1.2 mL of phosphate buffer, 0.1 mL of the stomach homogenate was added. The enzyme reaction was started by the addition of 1 mL of hydrogen peroxide solution. The change in the optical density was measured at 240 nm for 3 min at 30 s interval. Catalase activity is expressed as n moles of H_2O_2 utilized/min/mg protein in stomach homogenate[26].

Estimation of lipid peroxidation (LPO): Lipid peroxidation was assayed by using the following reagents: 8.1 % sodium dodecyl sulphate (SDS); 0.8 % thiobarbituric acid (TBA); 20 % acetic acid; distilled water; 15:1 v/v *n*-butanol: pyridine mixture.

The reaction mixture containing 0.2 mL of stomach homogenate, 1.5 mL of TBA, 0.2 mL of SDS, 1.5 mL of acetic acid and 0.8 mL of distilled water. The above reaction mixture is kept in boiling water bath at 90 ºC for 1 h and cooled in tap water. After cooling 1 mL of distilled water and 5 mL of a mixture of *n*-butanol and pyridine (15:1 v/v) were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance was read at 532 nm. The lipid peroxide concentration was expressed as n moles of MDA liberated/min/mg protein in stomach homogenate[27].

Measurement of gastric wall mucus content (GWMC): One half of the glandular portion of the stomach, opened along the greater curvature, was carefully separated from the rumenal part and transferred into 10 mL alcian blue 0.1 % (w/v) solution (alcian blue was dissolved in 0.16M sucrose buffered with sodium acetate 0.05M and finally adjusted to pH 5.8 with HCl 1M). The tissue was stained for 2 h in alcian blue solution; excess dye was removed by 2 successive rinses, soaking the tissue each time in 10 mL sucrose 0.25M, first for 15 min and then for 45 min. Dye complexed with gastric wall mucus was then extracted with 10 mL magnesium chloride at 0.5 h intervals for 2 h. Four mL of the extract was shaken with an equal volume of ether until an emulsion was formed. This was centrifuged at 3600 rpm for 10 min. Ether was pipetted out and discarded and the concentration of alcian blue was determined, in the aqueous layer. Colour absorbance was recorded using a spectrophotometer at 598 nm. The quantity of alcian blue extract per g wet glandular tissue was calculated[28].

RESULTS AND DISCUSSION

The results of physical and physico-chemical analysis of arista were tabulated in Table-1. The primary organic analysis on the both ethanolic extract of the crude drug (Patha) as well as the arista 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) (Table-2). An organoleptic analysis was also carried out on the arista and the results were tabulated (Table-3).

It is noteworthy and deserves a mention here that the ethanolic extract of *Viburnum* species has been proven to possess a remarkable antioxidant, antiinflammatotry and antiulcer activities. However, this drug, so far, has not been formulated in to any form and standardized for its value[29,30]. The arista itself and the arista added with water, 80 % methanol and ethyl acetate were observed under UV radiation showing greenish brown, brown, yellowish brown and pale brown colouration, respectively.

A primary organic analysis conducted on the arista 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 arista was greenish brown in colour; aromatic in odour; aromatic and sweet in taste; sticky after minutes in texture between fingers; pourable and non-sticky in nature to view; it turned brownish green 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

TABLE-1
STANDARDIZATION OF ARISTA BY PHYSICAL AND PHYSICO-CHEMICAL METHODS

Parameters	Report/values
Total solids	41.8 ± 0.15% w/mL
Boiling range	74 ± 0.04 – 107 ± 0.06 ºC
Congealing point	59 ± 0.008 – 67 ± 0.06 ºC
Content of ethanol	22% v/v at 32 ºC
Freezing point	9 ± 0.08 ºC
Loss on drying	19.82 ± 0.50 % w/w
Loss on Ignition	3.5 ± 0.33 % w/v
pH	4.8
Refractive Index against water (1.332)	1.325
Viscosity against water (0.9982)	1.9775 poise at 32 ºC
Weight per mL	1.102 g/mL
Total free sugar content	22 g % w/mL
Fluorescence analysis (long UV)	
(a) Arista	Greenish brown
(b) Arista in water	brown
(c) Arista with methanol	Yellowish brown
(d) Arista with ethylacetate	Pale brown

Results are presented as mean ± standard deviation, n = 3.

TABLE-2
PRIMARY ORGANIC ANALYSIS OF ARISTA AGAINST PATHA

Phytoconstituents	Arista	75 % ethanolic extract of patha
Carbohydrate	+++	++
Free sugar	+++	++
Amino acid	++	+
Alkaloid	–	–
Saponins	++	+
Phyto-sterols	–	–
Triterpenoids	–	–
Tannins	+++	++
Flavonoids	+++	++
Glycosides (general)	++	++
Glycoside (specific) (phenolic glycosides)	+++	+++
Anthocyanins	+++	+++

+: Test positive, –: Test negative.

TABLE-3
ORGANOLEPTIC ANALYSIS OF ARISTA

Parameters/characters	Results
Colour	Greenish brown
Odour	Aromatic
Taste	Ethanolic and Sweet
Texture	Sticky after minutes
Nature	Pourable, Non-sticky
Colour change at room temperature	Darkening when volume reduced
Odour upon heating	Ethanolic and pleasant

constant weight. The total solid of the arista were determined to be 41.8 ± 0.15 % 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 arista is concerned. In this event, the arista showed 74 ± 0.09-107 ± 0.06 ºC 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 arista, in this case, showed 59 ± 0.08-67 ± 0.06 ºC as the congealing point. Making no modification in the setting of apparatus the freezing point of the arista was determined to be 9 ± 0.08 ºC.

Since the principle behind the formulation of arista is that conversion of sugar (jaggery) in to ethanol by anaerobic fermentation process, determination of total alcohol concentration was determined to be 22 % v/v at 32 ºC 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 arista was determined to be 19.82 ± 0.50 % 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 arista in percentage w/v as determined to be 3.5 ± 0.33 % w/v. To determine the acidity or alkalinity of the arista, pH value was determined to be 4.8 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 arista using as Abbe refractometer against water[25] was measured to be 1.3.

By employing an Oswald-type viscometer, viscosity was determined against water to be 1.9775 poise at 32 ºC. Since arista is a liquid formulation, by using a calibrated pycnometer, the weight per mL of the arista was determined to be 1.102 g/mL at room temperature. The total free sugar content using Benedict's reagent for quantitative analysis was determined to be 22 %.

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 300 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 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 300 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 300 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 300 mg/kg dose showed a significant decrease ($p < 0.001$) in lipid peroxidation 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 300 mg/kg showed significant ($p < 0.05$) increase in gastric wall mucous content when compared to that of the solvent control (Table-4).

Probable mechanism of actions behind the biological activities of Triple *Viburnum* leaf 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.

Conclusion

A Triple *Viburnum* leaf arista was prepared and scrutinized for its antiulcer potential after its standardization some physicochemical methods. In water immersion plus restraint stress induced gastric ulcer model on rats, the status of all the biochemical parameters in the gastric juice, support the *Viburnum* arista treated animals to possess markable ulcer healing effect against stress induced ulcer when compared to that of their respective solvent control.

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TABLE-4
EFFECT OF *V. coriaceum* 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 mol of H_2O_2 consumed/ min/mg protein	Lipid peroxidation (n mol 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: #- $p < 0.05$, *-$p < 0.001$.

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