Natural Product Sciences
28(3) : 130-137 (2022)
https://doi.org/10.20307/nps.2022.28.3.130

Phytochemical Characterization of *Vitex negundo* Leaves: a Potent Antiandrogenic and Antioxidant Agent

Jayapal Sharath[1], Rafi Ahmed Shahin Taj[2], and Mahadevaiah Bhagya[3]*

[1]*Department of Studies in Zoology, Research scholar, University of Mysore, Mysuru, Karnataka, India*
[2]*Department of Studies in Zoology, Research scholar, University of Mysore, Mysuru, Karnataka, India*
[3]*Department of Studies in Zoology, Professor, University of Mysore, Mysuru, Karnataka, India*

Abstract – This study was conducted to characterise phytochemicals and to explore the biological activities of *Vitex negundo* leaves. The washed, course powder of *V. negundo* leaves were extracted with different solvents of increasing polarity. All the extracts were characterized and biological activities were compared. The results revealed that the ethanolic and cold water extracts showed the presence of all phytochemicals studied except protein compared to other extracts. Further, the quantitative estimation of phytochemicals showed that the ethanolic extract had highest yield and maximum amount of total polyphenols, flavonoids, and alkaloids with the least amount of tannins compared to other extracts studied. Furthermore, the highest total polyphenol content corresponds with the potent biological activities. Indeed, *in vitro* antioxidant and antisteroidogenic activities were highest in the ethanolic extract than others. To conclude, the present study is the first to report the characterization and antiandrogenic property of *V. negundo* leaf extracts. The ethanolic extract of *V. negundo* leaves can be used as an antioxidant and antiandrogenic agent. Hence, it can be considered for the treatment of hyperandrogenic conditions like polycystic ovary syndrome, etc.
Keywords – Antiandrogenic, antioxidant, polyphenols, five-leaved chaste tree, *in vitro*

Introduction

Phytochemicals are chemicals derived from plant origin. They are the chemicals produced by the plants through primary and secondary metabolism. They generally have biological activity in the host plant and play an essential role in the plant growth and defence against predators/ pathogens.[1] The phytochemicals have beneficial properties even in animals when administered, and hence can exploit these properties to enhance human health status. Among several phytochemicals, polyphenols and flavonoids are usually recommended as supplements for their rich antioxidant properties.[2] In addition, India with rich vegetation/ herbs and Ayurvedic system of medicine, provide additional benefit for the use of herbs to cure several diseased conditions. Further, recently pharmacological companies are relying on these phytocomponents to develop potent drugs. Hence, there is a dire need to study the phytochemicals present in different parts of the plant to employ the same for various diseased conditions by the pharmaceutical companies.

Vitex negundo Linn. is commonly known as the Chinese chaste tree, five leaved chaste trees belonging to the family Verbenaceae. Although all parts of *V. negundo* are used as medicine in the indigenous system of medicine, the leaves possess the most medicinal potency among them.[3] Traditionally, different parts of the plant and whole plant are used as medicinal plants as they have various medicinal values. To cite a few, of *V. negundo* leaves, fruits, roots, and seeds show different activities viz., the fresh leaves are used for the treatment of rheumatism, fever, pain, inflammation, skin diseases and the leaves, root, as well as bark, are used in snake bite cure.[4] The root is used as a tonic, expectorant, febrifuge and diuretic. Further, the flowers are used in the treatment of diarrhoea, cholera, and liver disorders.[5] In addition, anti-inflammatory and anti-arthritic,[6-10] immuno-stimulant,[11] anti-androgenic,[12-13] insecticidal and pesticidal,[14-15] anti-snake venom,[16] antioxidant[17,18] activities of *V. negundo* are reported by earlier studies. Further, it is reported that the leaves of *V. negundo* has analgesic,[19] hepatoprotective,[20] mosquito repellent,[15] antioxidant,[21,22] anti-estrogenic,[23] and anti-HIV[24] activities.

*Author for correspondence
Dr. Bhagya M, Professor, Department of Studies in Zoology, University of Mysore, Mysuru, India - 570006
https://orcid.org/0000-0003-1292-019X
E-mail: mbhagyauom@gmail.com

Although various biological properties of different parts, including those of leaves, are reported, the antiandrogenic property of the *V. negundo* leaves are not studied and reported. Furthermore, since it is known that secondary metabolites of the plants play different health beneficiary roles in the animal system, it is also necessary to understand the phytochemistry of the plant parts. Hence, the present study aimed to investigate the different phytoconstituents and the anti-androgenic activity by subjecting the leaves of *V. negundo* to sequential extraction with solvents based on increasing polarity so that the polar and nonpolar components can be separated and the biological activity of each can be understood.

Experimental

Collection and identification of plant materials – The *V. negundo* was collected from the Chandravana, Botanical garden maintained by the Mysore Medical College, Mysuru. An expert from the Department of Studies in Botany, University of Mysore, Mysuru-06, authenticated the herb.

Extract preparation – The leaves of *V. negundo* were separated from other parts of the plant. Further, washed the leaves in distilled water and rinsed in 70% alcohol to remove the mud and dirt if any, shade dried at room temperature and coarsely powdered. The powdered leaf material is subjected to sequential extractions with various solvents of increasing polarity, i.e., petroleum ether, benzene, chloroform, ethanol with respective temperature based on solvents using a soxhlet apparatus and cold water (4ºC for 24 h) and hot water (80ºC for 24 h) extracts. Then the extract was flash evaporated to eliminate the respective solvents completely, the yield of the extract was noted and was stored in 4ºC until further use.

The phytochemical analyses of *V. negundo* leave qualitatively – The different solvent extracts were individually analyzed for various phytoconstituents following the standard protocols.[25]

The phytochemical estimation of *V. negundo* leaves quantitatively – The quantitative analysis of total polyphenols, flavonoids, tannins, and alkaloids were determined following the standard protocols as briefed below.

Total phenolic content – The Folin-ciocalteu method[26] was employed to estimate the total phenolic content in different solvent extracts. About 1 mg of different plant extracts were dissolved in 1 mL of distilled water. The different concentrations of extracts were made up to 1 mL, to which added about 5 mL of 1:10 diluted Folin-ciocalteu reagent and 4 mL of sodium carbonate. The aliquot was mixed well and incubated at 45ºC for 10 minutes and read at 765 nm. A different concentration of gallic acid was used to prepare a standard calibration curve, calculated and expressed as mg gallic acid equivalent.

Total flavonoid contents – Estimated the flavonoid content following Chang et al.[27] Briefly, about 1 mg of each solvent extract was dissolved in 1 mL of distilled water. Further, the volume of different concentrations of extracts was made up to 4 mL with distilled water. To the reaction mixture, 0.3 mL of 5% (w/v) sodium nitrite was added. After 5 minutes, 0.3 mL of 10% aluminium chloride solution was added. Furthermore, 6 minutes post aluminium chloride addition, 2 mL of 1M sodium hydroxide solution was added to stop the reaction, and made up the volume to 10 mL with distilled water. The absorbance of the reaction aliquot was read at 510 nm and expressed as mg catechin equivalent.

Tannin content – The method described by Herald et al.[28] was followed to estimate the tannin content in different solvent extracts of *V. negundo* leaves. Briefly, the different solvent extracts of the leaves were incubated separately with the vanillin-HCl reagent in the dark for 20 minutes, and the absorbance was read at 500 nm. The results obtained were expressed as mg catechin equivalent / 100 g leaf sample.

Alkaloid content – The alkaloid content in different solvent extracts of *V. negundo* leaves was estimated according to Fadhil et al.[29] Briefly, the known amount of different solvent extracts was dissolved in 2N HCl separately and filtered. An equal volumes of phosphate buffer of pH 4.7 and bromocresol green solution was added to the filtrate. Then, chloroform was added to the content and shaken. Collected the extracted content in a volumetric flask, and the volume was made up to the mark with chloroform. The content in the volumetric flask was read at 470 nm against the blank. The results were expressed as mg atropine equivalent/ 100 g leaf sample.

Fourier-transform infrared (FTIR) spectroscopy analysis of *V. negundo* leaf extracts – The functional groups present in the different solvent extracts of *V. negundo* leaves were determined by FTIR analysis to determine the. Briefly, FTIR spectra was recorded at a resolution of 2 cm^{-1} (Tensor II, BrukerOptik GmbH, Germany) in the range of 400-4000 cm^{-1}.

2,2-Diphenyl-l-picryl hydrazine (DPPH) radical scavenging activity – The *in vitro* DPPH assay was conducted to determine the antioxidant potential of different leaf extracts of the herb *V. negundo* according to the method of Kalpna et al.[30]

***In vitro* ovarian steroidogenic enzyme activity** – The

ovarian steroidogenic enzyme inhibitory efficacy of different solvent extracts of *V. negundo* leaves was determined employing the ovarian 3β- and 17β- hydroxysteroid dehydrogenase (HSDH) as steroidogenic enzymes.[31] Briefly, 50 μg of different solvent extracts were incubated with the reaction aliquot for 1 hr at 37ºC and read at 490 nm.

Statistical analysis – All the estimations were carried out in triplicate, and the mean value of each parameter was computed. One way ANOVA was used to analyze and compare the mean values and judged significant if $P < 0.05$.

Results and discussion

The results of qualitative phytochemical analysis of different solvent extracts of the *V. negundo* leaves are listed in Table 1. The phytochemical analyses results revealed that the ethanolic and cold water extracts of the *V. negundo* leaves showed the majority of phytoconstituents followed by benzene and hot water extracts whereas petroleum ether and chloroform extracts showed the least number of phytoconstituents. Tannins, total polyphenols, alkaloids and steroids were present in all the extracts. Flavonoids were absent in petroleum ether, but saponins were present in petroleum ether, ethanol and cold water extracts. Proteins were absent in all the extracts tested, however, carbohydrates were present in all the extracts except petroleum ether and chloroform extracts.

The ethanolic extract (14.06%) of *V. negundo* leaves showed the highest percentage of yield, followed by cold water (8.92%), petroleum ether (6.2%), hot water (2.68%) and chloroform (1.49%) extracts. The benzene extract (0.39%) showed the very least percentage of yield compared to other extracts studied (Table 2).

The ethanolic extract (1650.55 ± 205.51 mg/100 g sample) showed significantly highest total polyphenolic content compared to other extracts. The ethanolic extract was followed by cold water (578.23 ± 23.57 mg/100 g sample), hot water (177.71 ± 59.79 mg/100 g sample), and petroleum ether (110.70 ± 32.86 mg/100 g sample) extracts respectively, with higher total polyphenol content. Further, the polyphenolic content is found to least in chloroform (82.71 ± 12.30 mg/100 g sample) and benzene (10.32 ± 3.00 mg/100 g sample) extracts compared to other extracts of *V. negundo* leaves (Table 2).

The flavonoid was absent in the petroleum ether extract and present in the least amount in benzene extract (210.39 ± 25.55 mg/100 g sample) compared to other extracts. Further, the ethanolic extract (7428.27 ± 918.59 mg/100 g sample) showed a significantly higher flavonoid content.

Table 1. Qualitative estimation of phytochemicals from different solvent extracts of the herb, *V. negundo* leaves

Bioactives/ Solvent extracts	Total polyphenols	Flavonoids	Alkaloids	Tannins	Saponins	Carbohydrates	Proteins	Steroids
Petroleum ether	+	-	+	+	+	-	-	+
Benzene	+	+	+	+	-	+	-	+
Chloroform	+	+	+	+	-	-	-	+
Ethanol	+	+	+	+	+	+	-	+
Cold water	+	+	+	+	+	+	-	+
Hot water	+	+	+	+	-	+	-	+

Table 2. Quantitative determination of phytochemicals and extract yield in different solvent extracts of *V. negundo* leaves

Solvent extracts	Percentage yield (%)	Total polyphenols (mg GAE / 100 g sample)	Flavonoids (mg CE/ 100 g sample)	Tannins (mg CE/ 100 g sample)	Alkaloid (mg AE/100 g sample)
Petroleum ether	6.2	110.70 ± 32.86^{a}	-	530.04 ± 77.92^{c}	362.41 ± 19.92^{c}
Benzene	0.39	10.32 ± 3.00^{a}	210.39 ± 25.55^{a}	116.88 ± 11.22^{a}	24.57 ± 0.87^{a}
Chloroform	1.49	82.71 ± 12.30^{a}	1013.24 ± 79.81^{a}	432.02 ± 50.24^{c}	548.62 ± 1.46^{d}
Ethanol	14.06	1650.55 ± 205.51^{c}	7428.27 ± 918.59^{c}	61.62 ± 15.53^{a}	1351.16 ± 82.49^{e}
Cold water	8.92	578.23 ± 23.57^{b}	4813.64 ± 954.32^{b}	$374.09 \pm 112.96^{b,c}$	$469.91 \pm 31.02^{c,d}$
Hot water	2.68	177.71 ± 59.79^{a}	1184.78 ± 203.69^{a}	$209.89 \pm 3.64^{a,b}$	222.37 ± 8.07^{b}
Significance	-	P<0.001	P<0.001	P<0.003	P<0.001

Note: All the values are mean ± SEM

Mean values with same superscript letters in the given column are not significantly different, whereas those with different superscript letters are significantly ($P < 0.05$) different as judged by Duncan's post-hoc test. AE = Atropine equivalent; GAE = Gallic acid equivalent; CE = Catechin equivalent

The cold water (4813.64 ± 954.32 mg/100 g sample), hot water (1184.78 ± 203.69 mg/100 g sample) and chloroform (1013.24 ± 79.81 mg/100 g sample) extracts followed the ethanolic extract respectively for the highest flavonoid content (Table 2).

The amount of tannin in different solvent extracts was determined. The results revealed that the ethanolic extract (61.62 ± 15.53 mg/100 g sample) showed significantly less amount of tannins followed by benzene (116.88 ± 11.22 mg/100 g sample), hot water (209.89 ± 3.64 mg/100 g sample) and cold water (374.09 ± 112.96 mg/100 g sample) extracts respectively. However, petroleum ether (530.04 ± 77.92 mg/100 g sample) and chloroform (432.02 ± 50.24 mg/100 g sample) extracts showed high tannin content compared to other extracts (Table 2).

The ethanolic extract (1351.16 ± 82.49 mg/100 g sample) reported the maximum alkaloid content compared to other extracts. Chloroform (548.62 ± 1.46 mg/100 g sample), cold water (469.91 ± 31.02 mg/100 g sample), petroleum ether (362.41 ± 19.92 mg/100 g sample), and hot water (222.37 ± 8.07 mg/100 g sample) extracts of *V. negundo* leaves showed alkaloids content in the order mentioned followed by the ethanolic extract. The benzene extract (24.57 ± 0.87 mg/100 g sample) showed the very negligible amount of alkaloid compared to other extracts (Table 2).

The presence of functional groups in different solvent extracts of *V. negundo* leaves was determined by FTIR analysis (Fig. 1). The FTIR spectra of ethanol extract showed a strong, broad peak at 3319 cm^{-1}, indicating the presence of the -OH group. In addition, the benzene extract showed medium sharp stretch at 3627 cm^{-1} and 3588 cm^{-1}, whereas chloroform and cold water extracts showed a weak broad peaks at 2927 $cm^{-1}$and 3177 cm^{-1} respectively for –OH, suggesting the alcoholic group. Further, peaks at 3307 cm^{-1} and 3272 cm^{-1} for –NH indicates aliphatic primary amine, medium stretching peak at 2942 cm^{-1} for –CH indicates alkane group, weak peaks at 2200 cm^{-1} for C≡C suggests disubstituted alkyne, and at 1979 $cm^{-1}$for –CH suggests an aromatic compound in petroleum ether extract. Similarly, benzene extract showed medium sharp –NH peak at 3273 cm^{-1} for aliphatic primary amine, weak broad –OH stretch at 2941 cm^{-1} for intra-molecular bonded alcohol, a weak peak at 2200 cm^{-1} for C≡C suggests disubstituted alkyne and a weak bending at 1999 cm^{-1} indicates aromatic compound. The chloroform extract showed medium –NH stretch at 3323 cm^{-1} for aliphatic primary amine, medium –CH stretch at 2850 cm^{-1} for alkane and strong stretches at 1005 cm^{-1} and 678 cm^{-1} for fluoro and halo (C-I) compounds, respectively. In addition to the broad –OH peak, the ethanolic extracts showed weak monosubstituted alkyne (C≡C) stretch at 2118 cm^{-1}, medium stretch for alkene (C=C) at 1636 cm^{-1}

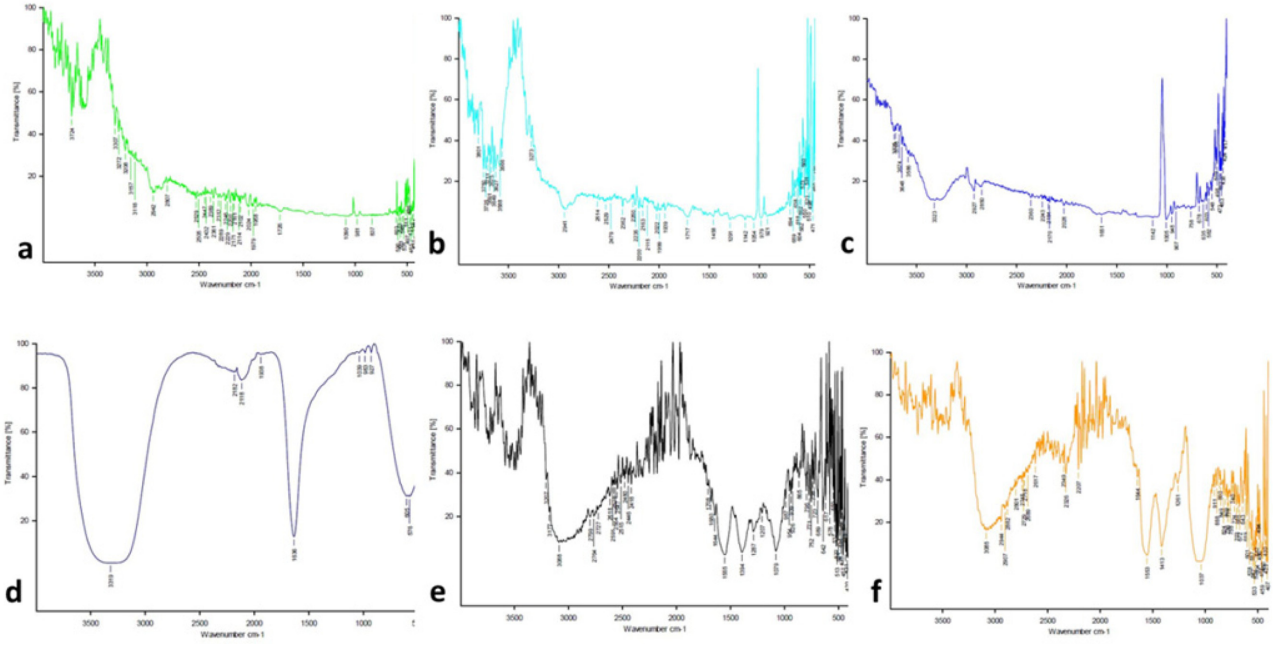

Fig. 1. FTIR spectra of (a) petroleum ether (b) benzene (c) chloroform (d) ethanolic (e) cold water and (f) hot water extracts of *V. negundo* leaves showing bands of different functional groups.
Note the broad band at the range of 3600-3000 cm^{-1} in (d) spectra suggesting the presence of -OH group.

Table 3. DPPH scavenging activity of standard and different solvent extracts of *V. negundo* leaves

Extracts	IC_{50} value (μg/mL)
Standard	131.05 ± 0.25
Petroleum ether	32.20 ± 1.05[b]
Benzene	35.68 ± 0.42[c]
Chloroform	37.60 ± 1.44[c]
Ethanol	23.05 ± 0.54[a]
Cold water	24.48 ± 0.66[a]
Hot water	29.99 ± 1.99[b]

Note: All the values are mean ± SEM
Mean values with same superscript letters in the given column are not significantly different, whereas those with different superscript letters are significantly ($P < 0.05$) different as judged by Duncan's post-hoc test.

and a strong stretch at 605 cm^{-1} for the halo (C-Br) group. Furthermore, weak disubstituted alkyne stretch at 2207 cm^{-1}, medium cyclic alkene (C=C) stretch at 1553 cm^{-1}, and a medium alkane (C-H) bend at 1413 cm^{-1} were found in the hot water extract. A strong, broad stretch of –OH for carboxylic acid at 3088 cm^{-1}, medium stretches at 1644 cm^{-1}, and 1555 cm^{-1} for conjugated alkene and a cyclic alkene with a medium bend for alkane (-CH methyl group) at 1394 cm^{-1} was found in cold water extract spectra.

The DPPH radical scavenging assay results revealed that all the solvent extracts of *V. negundo* studied in the present work have potent free radical scavenging activity. They have a lesser IC_{50} value than to that of standard ascorbic acid. The ethanol and cold water extracts were more potent in scavenging the free radicals than other solvent extracts with least IC_{50} values, i.e., 23.05 ± 0.54 μg/mL and 24.48 ± 0.66 μg/mL respectively compared to other extracts and standard ascorbic acid (131.05 ± 0.25 μg/mL) (Table 3).

The ovarian 3β- and 17β-HSDH activities were decreased by all the six solvent extracts studied. However, the ethanolic extract of *V. negundo* leaves significantly reduced the activities of ovarian 3β-HSDH (Fig. 2) and 17β-HSDH (Fig. 3) compared to control.

The secondary metabolites of the naturally available herbs are known to have health beneficial properties. The leaves of the herb *V. negundo* were subjected to different solvent extraction and were analyzed qualitatively to understand the presence of different phytoconstituents. The results revealed that the different leaf extracts of the herb showed the presence of polyphenols, flavonoids, alkaloids, tannins, saponins, carbohydrates and steroids but proteins were absent. Ethanolic extract consisted of almost all the phytoconstituents tested. In addition, the ethanolic extract showed maximum yield, followed by

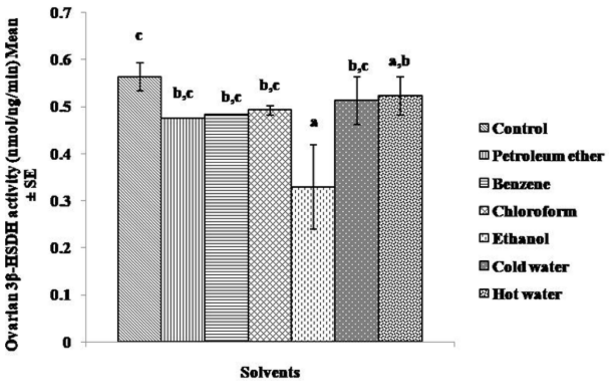

Fig. 2. Vertical bars showing the inhibitory action of *Vitex negundo* leaf extract on the activity of ovarian 3β-HSDH.
Note: All the values are mean ± SEM
Mean values with same superscript letters in the given column are not significantly different, whereas those with different superscript letters are significantly ($P < 0.05$) different as judged by Duncan's post-hoc test.

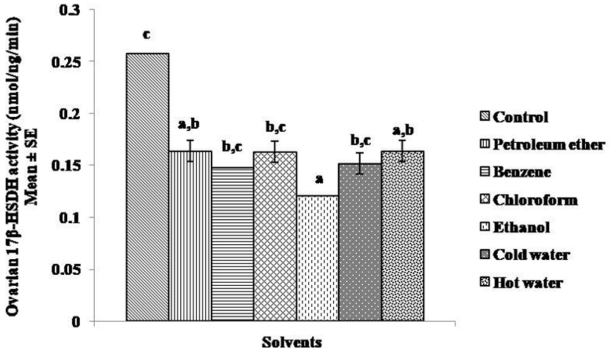

Fig. 3. Vertical bars showing the inhibitory activity of *V. negundo leaf* extract on the ovarian 17β-HSDH.
Note: All the values are mean ± SEM
Mean values with same superscript letters in the given column are not significantly different, whereas those with different superscript letters are significantly ($P < 0.05$) different as judged by Duncan's post-hoc test.

cold water, petroleum ether, hot water, chloroform and benzene extracts.

Further, the ethanolic extract showed the highest amount of total polyphenols, flavonoids and alkaloid content with the least amount of tannin. Furthermore, the biological properties of different solvent extracts studied were also determined. The antioxidant and antisteroidogenic activities were most significant in the ethanolic extract, followed by cold water extract. These biological properties may be due to the highest concentration of polyphenols, as it is well established that the polyphenols showed high antioxidant activity. Indeed in the present study, the leaf extract of *V. negundo* showed potent

antioxidant and antisteroidogenic properties/activities, with ethanolic extract showing the highest activity compared to others.

Mankind is facing/suffering from several diseases due to various factors like environmental (heat, cold, light, etc.), chemicals (pesticides, cosmetics), psychological (stress, fear) etc. Several synthetic drugs are being used to manage the conditions. However, in addition to their beneficiary property, synthetic drugs have side effects, affecting the human system from simple nausea and fever to death. Hence, naturally available herbs as medicine are a better alternative as they have little or no harmful effects on the human system.

Herbs can be used to fight various diseased conditions in humans because, similar to humans, plants also get exposed to harsh environments/conditions, and to fight against these conditions, plants synthesize and produce secondary metabolites.[32] These secondary metabolites have health beneficial properties in animals/humans.[33] For instance, *Curcuma longa*, *Withania somnifera*, *Zingiber officinale, Acorus calamus*, *Tinospora cordifolia*, *Carica papaya, Hemidesmus indicus*, etc., are used as anti-inflammatory,[34,35] anti-diabetic,[36,37] cardioprotective,[38] anti-obesity,[39] anti-microbial,[40,41] and anti-stress[42] agents.

V. negundo is one such natural herb with medicinal value. To cite a few, *V. negundo* has anti-inflammatory,[43] antinociceptive,[44] enzyme-inhibitory,[45] antibacterial,[46] anti-allergic,[8] snake venom neutralization,[16] hepatoprotective,[47] laxative,[48] immunomodulatory,[49] insecticidal and pesticidal,[14,15,50] analgesic,[51,52] anti-microbial,[3] anti-bacterial,[53] and HIV type 1 reverse transcriptase inhibitory[24] activities. Despite several studies showing various health beneficiary properties, the phytochemical characterization of *V. negundo* leaves is not reported. Hence, it is necessary to understand the presence and quantity of secondary metabolites in the herb or its parts and explore its biological properties.

In the present study, the leaves were subjected to sequential extraction with different solvents based on increasing polarity with its respective temperature, i.e., petroleum ether, benzene, chloroform, ethanol, cold water, and hot water to separate polar and non-polar compounds. Further, the extract yield was noted and analyzed the phytochemical presented qualitatively and quantitatively. The qualitative analyses showed the absence of flavonoids in petroleum ether extract. Carbohydrates were absent in petroleum ether and chloroform extracts, whereas, saponins was absent in benzene and chloroform extracts. The steroids and the tannins were present, but proteins were absent in all the solvent extracts studied. This differential distribution of bioactives may be due to the sequential extraction method.

Further, the results of the quantitative analyses revealed that the ethanolic extract showed the highest concentration of polyphenols, flavonoids, and alkaloids, followed by the cold water extract. The least amount of total polyphenols was found in benzene extract. This may be because benzene is a non-polar solvent and extracts non-polar substances, but ethanol and cold water are polar solvents and extracts polar substances in higher amounts. The ethanolic extracts of *V. negundo* leaves showed the least amount of tannin, with petroleum ether showing the maximum amount of tannin present. Though tannins are known to have health beneficial properties like antitumor, antibacterial and antiviral,[54] it is also known to have anti-nutritional value.[55] Hence, ethanolic extract with the highest concentration of polyphenols, flavonoids and alkaloids and the least amount of tannin than other extracts studied suggests that ethanolic extract with rich bioactives and least anti-nutritional factor can be the best health beneficial agent.

It is clear from the above discussion that the ethanolic extract of *V. negundo* leaves has maximum phytochemicals. However, this should also be substantiated with its biological properties. Hence, the antioxidant property of the extracts studied was analyzed *in vitro* by DPPH assay. The DPPH assay is a widely used *in vitro* to determine the free radical scavenging property. The DPPH is a stable free radical. It accepts electron reduces in the presence of antioxidants and becomes 2, 2-diphenyl-l-picryl hydrazine,[56] which is read at 517 nm. Several studies reported that polyphenols show potent antioxidant efficacy.[57] Indeed, in the present study, the ethanolic and cold water extracts with the highest concentration of polyphenols had the most potent antioxidant efficacy as the minimum amount of about 23.05 ± 0.54 μg/mL extract inhibited about 50% of DPPH. The present study reports a very minimal amount of extract to inhibit 50% of DPPH compared to earlier studies.[58,59] The hot water, petroleum ether, benzene, chloroform extracts showed antioxidant potency, followed by ethanolic and cold water extracts in the order mentioned.

Further, in addition to the antioxidant property, the anti-steroidogenic property was also determined. It is well established that hyperandrogenism is a characteristic feature of several pathological conditions, like polycystic ovarian syndrome, cancer, endometriosis, stress, etc. The steroidogenic enzymes, 3β-HSDH and 17β-HSDH, play a vital role as they are rate-limiting steps in the steroidogenesis /synthesis of steroid hormones. The steroidogenic

hormone testosterone, is present in a very small amount in females. However, under pathological conditions, the testosterone level elevates and impairs female reproductive processes. Hence, suppression of 17β-HSDH activity would suppress the synthesis of testosterone, and the condition can be managed to normal. Similar to an antioxidant property, the ethanolic extract suppressed the activities of both steroidogenic enzymes efficiently than the control and other extracts studied. Hence, ethanolic extract can be used as an antiandrogenic agent, and this is the first study to report the antiandrogenic property of *V. negundo* leaf. Although, it was reported earlier that *V. negundo* seed has antiandrogenic property,[60] the leaf would be better to use as an antiandrogenic agent than seed of *V. negundo* as a higher amount of its seed oil is reported be toxic.

To conclude, the sequential extraction of *V. negundo* leaves showed the differential distribution of phytochemicals in different solvent extracts. The extraction using a polar solvent, i.e., ethanol, extracted the maximum amount of polyphenols, flavonoids and alkaloids and exhibited potent antioxidant and anti-steroidogenic properties. Hence, ethanolic extract can be used as an antioxidant and anti-steroidogenic agents upon further confirmation in an animal model.

Acknowledgement

We thank University of Mysore for the award of fellowship to the first author University SC/ST special cell fellowship scheme. We would also thank University of Mysore for the equipment facility provided in the Institution of Excellence.

References

(1) Mazid, M.; Khan, T. A.; Mohammad, F. *Biol. Med.* **2011**, *3*, 232-249.
(2) Pandey, K. B.; Rizvi, S. I. *Oxid. Med. Cell. Longev.* **2009**, *2*, 270-278.
(3) Keerti, G.; Padma, K. *Int. J. Drug Dev. Res*. **2012**, *4*, 192-199.
(4) Bano, U.; Jabeen, Z.; Ahmed, A.; Siddiqui, M. A. *World J. Pharm. Res*. **2015**, *4*, 589-606.
(5) Warrier, P. K.; Nambiar, V. P. K. Indian medicinal plants: A compendium of 500 species. Vol. 5; Orient Longman Private Limited: India, **2002**, p 387.
(6) Chaturvedi, G. N.; Singh, R. H. *Indian J. Med. Res.* **1965**, *53*, 71-80.
(7) Chawla, A. S.; Sharma, A. K.; Handa, S. S.; Dhar, K. L. *Indian J. Chem*. **1991**, *30B*, 773-776.
(8) Chawla, A. S.; Sharma, A. K.; Handa, S. S.; Dhar, K. L. *J. Nat. Prod.* **1992**, *55*, 163-167.
(9) Utpalendu, J.; Chattopadhyay, R. N.; Shaw, B. P. *Indian J. Pharmacol.* **1999**, *3*, 232-233.
(10) Kulkarni, R. R.; Virkar, A. D.; D'mello, P. *Indian J. Pharm. Sci.* **2008**, *70*, 838-840.
(11) Singh, D. D.; Chitra, G.; Singh, I. P.; Bhutani, K. K. *Indian J. Chem.* **2005**, *44B*, 1288-1290.
(12) Bhargava, S. K. *J. Ethnopharmacol.* **1989**, *27*, 327-339.
(13) Samy, P. R.; Ignacimuthu, S.; Sen, A. *J. Ethnopharmacol.* **1998**, *62*, 173-182.
(14) Deshmukh, P. B.; Chavan, S. R.; Renapurkar, D. M. *Pesticides*, **1982,** *16*, 7-10.
(15) Hebbalkar, D. S.; Hebbalkar, G. D.; Sharma, R. N.; Joshi, V. S.; Bhat, V. S. *Indian J. Med. Res.* **1992**, *95*, 200-203.
(16) Alam, M. I.; Gomes, A. *J. Ethnopharmacol.* **2003**, *86*, 75-80.
(17) Zheng, G.; Luo, Z. Guangdong Huangong, **1999**, *2*, 8-9.
(18) Ono, M.; Nishida, Y.; Masuoka, C.; Li, J.; Okawa, M.; Ikeda, T.; Nohara, T. *J. Nat. Prod*. **2004**, *67*, 2073-2075.
(19) Dharmasiri, M. G.; Jayakody, J. R. A. C.; Galhena, G.; Liyanage, S. S. P.; Ratnasooriyab, W. D. *J. Ethnopharmacol*. **2003**, *87*, 199-206.
(20) Kadir, F. A.; Kassim, N. M.; Abdulla, M. A.; Yehye, W. A. *Evid. Based Complement Alternat. Med.* **2013**, *2013*, 739850.
(21) Tondon, V. R.; Gupta, R. K. *Indian J. Physiol. Pharmacol.* **2005**, *49*, 199-205.
(22) Tiwari, O. P.; Tripathi, Y. B. *Food Chem.* **2007**, *100*, 1170-1176.
(23) Jivarajani, M.; Ravat, N.; Anandjiwala, S.; Nivsarkar, M. *Int. Sch. Res. Notices* **2014**, *2014*, 241946.
(24) Kannan, M.; Rajendran, P.; Vedha, V.; Ashok, G.; Anushka, S.; Ramachandran Nair, P. C. *J. Cell Mol. Biol.* **2012**, *10*, 53-59.
(25) Mumtaz, F.; Raza, S. M.; Ahmad, Z.; Iftikhar, A.; Hussain, M. *J. Pharm. Altern. Med.* **2014**, *3*, 17-21.
(26) McDonald, S.; Prenzler, P. D.; Antolovich, M.; Robards, K. *Food Chem.* **2001**, *73*, 73-84.
(27) Chang, C. C.; Yang, M. H.; Wen, H. M.; Chern, J. C. *J. Food Drug Anal.* **2002**, *10*, 178-182.
(28) Herald, T. J.; Gadgil, P.; Perumal, R.; Bean, S. R.; Wilson, J. D. *J. Sci. Food Agric*. **2014**, *94*, 2133-2136.
(29) Fadhil, S.; Reza, M. H.; Rouhollah, G.; Reza, V. R. M. *Res. J. Phytochem.* **2007**, *1*, 79-82.
(30) Rakholiya, K.; Kaneria, M.; Chanda, S. *J. Med. Plants Res.* **2011**, *5,* 63-71.
(31) Shivanandappa, T.; Venkatesh, S. *Anal. Biochem.* **1997**, *254*, 57-61.
(32) Pagare, S.; Bhatia, M.; Tripathi, N.; Pagare, S.; Bansal, Y. K. *Curr. Trends Biotechnol. Pharm.* **2015**, *9*, 293-304.
(33) Farnsworth, N. R.; Akerele, O.; Bingel, A. S.; Soejarto, D. D.; Guo, Z. *Bull. World Health Organ.* **1985**, 63, 965-981.
(34) Ramadan, G.; Al-Kahtani, M. A.; El-Sayed, W. M. *Inflammation* **2011**, *34*, 291-301.
(35) Savaringal, J. P.; Lally, M. S. *Int. J. Basic Clin. Pharmacol.* **2018**, *7*, 229-233.
(36) Juarez-Rijop, I. E.; Diaz-Zagoya, J. C.; Ble-Castillo, J. L.; Miranda-Oscorio, P. H.; Castell-Rodriguez, A. E.; Tovilla-Zarate, C. A.; Rodriguez-Hernandez, A.; Aguilar-Mariscal, H.; Ramon-Fris, T.; Bermudez-Ocana, D. Y. *BMC Complement. Altern. Med.* **2012**, *12*, 236.
(37) Sharma, N.; Bano, A.; Dhaliwal, H. S.; Sharma, V. *Int. J. Pharm. Pharm. Sci.* **2015**, *7*, 30-34.
(38) Zarei, M.; Javarappa, K. K.; Zarei, M.; Baker, S. *Der Pharmacia Lettre* **2013**, *5*, 334-339.
(39) Kim, D. S.; Kim, S. H.; Cha, J. *Evid. Based Complement. Altern. Med*. **2016**, *2016*, 9735276.
(40) Jeyachandran, R.; Xavier, T. F.; Anand, S. P. *Anc. Sci. Life* **2003**, *23*, 40-43
(41) Gul, P.; Bakht, J. *J. Food Sci. Technol.* **2015**, *52*, 2272-2279.
(42) Sarjan, H. N.; Divyashree, S.; Yajurvedi, H. N. *Pharm. Biol*. **2017**, *55*, 1358-1367.
(43) Ahirrao, R. A.; Patel, M. R. *Asian J. Res. Chem*. **2012**, 5, 843-845.
(44) Zheng, C. J.; Huang, B. K.; Han, T.; Zhang, Q. Y.; Zhang, H.;

Rahman, K.; Qin, L. P. *Pharma. Biol.* **2010**, *48*, 651-658.
(45) Zhao, X. Y.; Wang, B. E.; Li, X. M.; Wang, T. L. *Pathol. Int.* **2008**, *58*, 580-588.
(46) Alshawsh, M. A.; Abdulla, M. A.; Ismail, S.; Amin, Z. A. *Evid. Based Complement. Alternat Med.* **2011**, *2011*, 103039.
(47) Mahalakshmi, R.; Rajesh, P.; Ramesh, N.; Balasubramanian, V.; Kannan, V. R. *Int. J. Pharmacol.* **2010**, *6*, 658-663.
(48) Dashti, H. M.; Mathew, T. C.; Jadaon, M. M.; Ashkanani, E. *Nutrition* **1997**, *13*, 206-212.
(49) Nakajima, M.; Iwata, K.; Yamamoto, T.; Funae, Y.; Yoshida, T.; Kuroiwa, Y. *Drug Metab. Dispos.* **1998**, *26*, 36-41.
(50) Prakash, A.; Mathur, K. C. *Bull. Grain Technol.* **1985**, *23*, 278-281.
(51) Ravishankar, B.; Bhaskaran, N. R.; Sasikala, C. K. *Bull. Medico-Ethno-Botanical Res.* **1985**, *6*, 72-92.
(52) Ravishankar, B.; Bhaskaran, N. R.; Sasikala, C. K. *J. Res. Ayurv. Siddha*. **1986**, *7*, 62-77.
(53) Kurapatti, P.; Murugesan, K.; Anbalagan, S.; Sankareswaran, M. *Int. J. Pharm. Sci. Rev. Res*. **2017**, *46*, 183-187.
(54) Ueda, K.; Kawabata, R.; Irie, T.; Nakai, Y.; Tohya, Y.; Sakaguchi, T. *PLoS ONE*, **2013**, *8*, e55343.
(55) Ashok, P. K.; Upadhyaya, K. *J. Pharmacogn. Phytochem*. **2012**, *1*, 45-50.
(56) Sumathy, R.; Sankaranarayanan, S.; Bama, P.; Ramachandram, J.; Vijayalakshmi, M.; Deecaraman, M. *Asian J. Pharm. Clin. Res.* **2013**, *6*, 211-214.
(57) Scalbert, A.; Johnson, I. T.; Saltmarsh, M. *Am. J. Clin. Nutr.* **2005**, *81*, 215S-217S.
(58) Lakshmanashetty, R. H.; Nagaraj, V. B.; Hiremath, M. G.; Kumar, V. *Chiang Mai J. Sci*. **2010**, *37*, 489-497.
(59) Zargar, M.; Azizah, A. H.; Roheeyati, A. M.; Fatimah, A. B.; Jahanshiri, F.; Pak-Dek, M. S. *J. Med. Plants Res.* **2011**, *5*, 2525-2532.
(60) Divya, K.S.; Swati, S.P. *Int. J. Pharm. Sci. Rev. Res.* **2015**, *33*, 211-216.

Received April 7, 2022
Revised June 19, 2022
Accepted July 29, 2022