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RESEARCH ARTICLE

Phytochemical Evaluation and Antioxidant Activity of *Holarrhena pubescens* Wall. ex G.Don

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ABSTRACT

The selected medicinal plant *Holarrhena pubescens* Wall. ex G.Don belongs to the family Apocynaceae, and it was collected in hillock of Muthu Malai hill in Coimbatore, Tamil Nadu. In the present study, preliminary phytochemical screening of *H. pubescens* a medicinal plant was carried out. Qualitative phytochemical analysis of these plants confirms the presence of various secondary metabolites such as steroids, tannins, alkaloids, and phenols. The results suggest that the phytochemical properties for curing various ailments possess potential anti-inflammatory, antimicrobial, and antioxidant and leads to the isolation of new and novel compounds. Gas chromatography-mass spectrometry analysis showed the existence of various compounds with different chemical structures. The presence of various bioactive compounds confirms the application of *H. pubescens* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug. Extracts from *H. pubescens* showed varying antioxidant (free radical scavenging) activities when compared to Vitamin C, and the results suggest that the antioxidant activity of *H. pubecens* may contribute to their claimed medicinal property.

Keywords: *Holarrhena pubescens,* Indian medicinal plants, Phytochemical screening

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities.[1] Many of these indigenous medicinal plants are used as spices and food plants. They are sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes.[2] Medicinal plants are generally used in traditional medicine for the treatment of many ailments Ogukwe *et al*., 2004).[3] Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter. Majority of the diseases/ disorders are mainly linked to oxidative stress due to free radicals. The free radicals (oxidants) are species with very short half-life, high reactivity, and damaging activity toward macromolecules such as proteins, DNA, and lipids. In general, the reactive oxygen species circulating in the body tend to react with the electron of other molecules in the body and these also affect various enzyme systems and cause damage which may further contribute to conditions such as cancer, ischemia, aging, adult respiratory distress syndromes, and rheumatoid arthritis.

A plant-based diet protects against chronic oxidative stress-related diseases. Dietary plants contain variable chemical families and amounts of antioxidants. It has been hypothesized that plant antioxidants may contribute to the beneficial health effects of dietary plants. *Holarrhena pubescens* belonging to family Apocynaceae commonly known as kutaja or kurchi (Malyalam, India) is distributed in Asia, tropical areas of Africa, Madagascar, India, Philippines, and Malayan Peninsula. *H. pubescens* growing up to an altitude of 1300m in the Himalayas. It gows often sociably in deciduous forests and open waste.[4] The plant has been employed for long time in folklore therapy. “Kurchi” bark is an important traditional drug used in various ailments. The drug is astringent, anthelmintic, stomachic, antipyretic, and tonic and is generally administered as an extract or decoction in amoebic dysentery and diarrhea. Bark is given either alone or with other

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astringent drugs in piles, colic, dyspepsia, chest affections, and diuretics and also reported to be useful in skin diseases and spleen. The present study is aimed to analyze the phytochemical analysis and antioxidant activity of *H. pubescens*.

MATERIALS AND METHODS

Collection of plant materials

The selected medicinal plant like *H. pubescens* was collected in hillock of Muthu Malai Murugan temple hill, in Kinathukadavu, Coimbatore district, Tamil Nadu.

Preparation of plant extracts

30 g of powdered *H. pubescens* leaf was successively extracted using 300 ml of methanol and petroleum ether using the Soxhlet extractor for 8–10 h.[5] The extract was filtered through Whatman No. 1 filter paper to remove all undissolved matter including cellular materials and other constitutions that are insoluble in the extraction solvent.

Preliminary phytochemical studies

The methanol and petroleum ether extracts were subjected to preliminary phytochemical tests to determine the group of secondary metabolites present in the powdered *H. pubescens* which was followed by Harborne.[6]

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of the ethanol extract of *H. pubescens* Wall. ex G.Don leaf was performed using Shimadzu Japan GC QP2010 plus with a fused GC column coated with polymethylsilicon (0.25 nm × 50 m) and the conditions were as follows: Temperature programming from 80 to 200°C held at 80°C for 1 min, rate 5°C/min, and at 200°C for 20 min. Field ionization detector temperature of 300°C, injection temperature of 220°C, carrier gas nitrogen at a flow rate of 1 ml/min, and split ratio of 1:75 GC-MS were conducted using GCMS-QP 2010 plus Shimadzu Japan with an injector temperature of 220° and carrier gas presence of 116.9 kpa. The color length is 30 m with a diameter of 0.25 mm and flow rate of 50 ml/min. Elutes were automatically passed into a MS with a dictator voltage set at 1.5 kv and sampling rate 0.2 s. The MS was also equipped with a computer fed mass spectra bank. German Hermlez 233M-Z centrifuge was used.

Antioxidant assay

2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity

The antioxidant activity of the methanolic extract of *H. pubescens* was measured on the basis of the scavenging activity of the stable DPPH free radical according to the method described by Brand-Williams *et al*.[7]

RESULTS

Preliminary phytochemical analysis of *H. pubescens*

In this phytochemical evaluation, initially physical constants were evaluated for its presence as well as for its quantity. The petroleum ether and methanolic extracts were found to contain flavonoids, saponins, glycosides, steroids, and phenolic compounds. The plant material was subjected to phytochemical analysis separately for observing the presence of alkaloids, flavonoids, terpenoids, phenolic compounds, glycosides, saponins, steroids, and tannin [Table 1]. All results observed were in leaves of *H. pubescens*. Flavonoids are found in optimum concentration in the present study. Flavonoids are pharmacologically active substances. Saponins are steroid glycosides. It may be steroid glycosides or may be terpene glycolysides. The combination of hydrophilic triterpene with a hydrophilic sugar gives saponins. In general, saponins are toxic, but many experiments showed that consumption of saponins in lower concentration by human beings may be beneficial in reducing heart diseases. In the present investigation because of the presence of saponins, the leaves of *H. pubescens* in methanolic solvent may have some medicinal property. Glycosides were also present in *H. pubescens*. The present study reveals optimum precipitation of glycosides. Hence, the plant may be tested for anti-stress, antidiabetic, and anti-inflammatory properties as is evident from the works of above-

mentioned authors. Phenolic compounds were also detected in both solvents. They show a high degree of precipitation of phenolic compounds.

GCMS analysis of *H. pubescens*

The plant extract of *H. pubescens* (methanol extract) *was* analyzed by GC-MS. The presence of components was confirmed by comparing mass spectra of analyzed components with standard mass spectra of NIST and Willey library. In the The identified compounds represented with Retention Time,Chemical Formula, Molecular weight, Peak area %, Structure and Medicinal Uses.

DPPH scavenging activity of *H. pubescens* Wall. ex G.Don

The antioxidant activities in leaf of *H. pubescens* methanol and ethanolic extracts were assessed by DPPH activity. The DPPH activity of different concentration of methanol and ethanolic extracts (50–250 µg/ml) along with standard ascorbic acid is presented in the [Table 3]. With the increasing concentrations, positive scavenging activity was noted. The percentage of scavenging activity is increasing with the increasing concentration in both extracts. Among the five different concentration (50–250 µg/ml) of ethanolic and Methanolic extracts tested, the ethanol extract showed higher inhibition (43.6 ± 0.88) was observed in 250 µg/ml concentration followed by (75.4 ± 0.86) 250 µg/ml of methanol extract against ascorbic acid (85.2 ± 0.92) The ethanol extract showed higher inhibition (40.6 ± 0.88) in 200 µg/ml concentration and methanol extract showed (72.1 ± 0.94) followed by the ascorbic acid showed (78.8 ± 0.92) in 200 µg/m concentration. From the result when comparing the scavenging activity percentage of ethanol and methanol, the methanol extract shows higher activity than ethanol extract. DPPH free radicals have the ability to take electron from the antioxidants that is why it is used for the antioxidant scavenging assays of the medicinal plants for its estimation. Table 3 shows the percentage scavenging activity in ethanol and

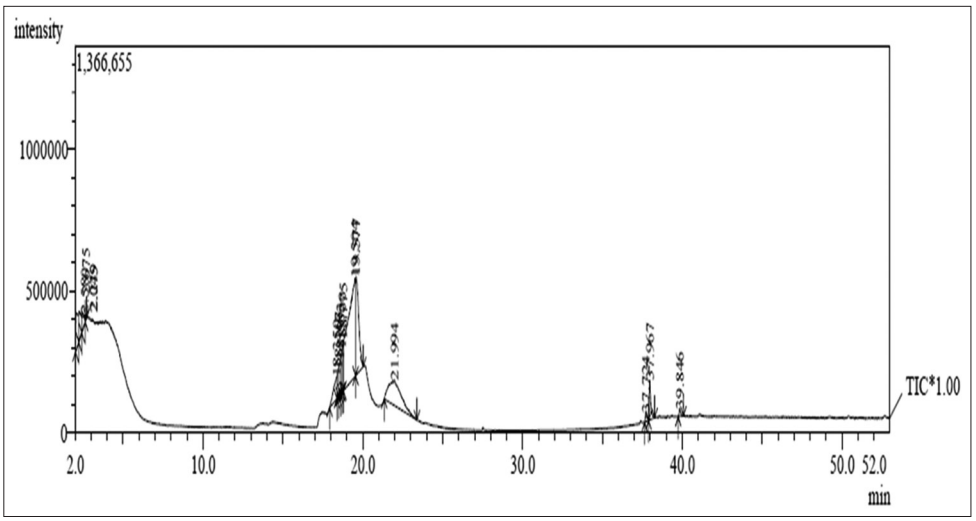


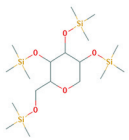
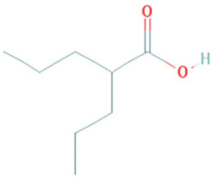
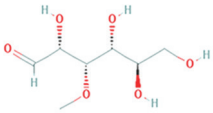
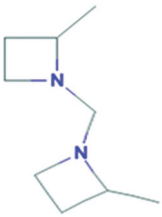
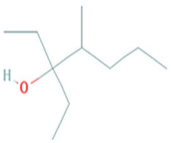
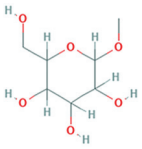
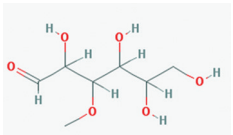
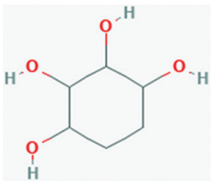
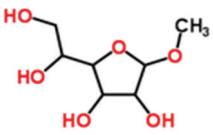
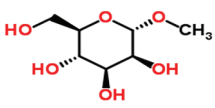
Figure 1: Gas chromatography–mass spectrometry analysis in methanolic extracts of *Holarrhena pubescen*

Table 1: Pliminary phytochemical analysis in leaf of *H. pubescens*

S. No	Name of the secondary metabolite	Petroleum ether	Methanol
1	Alkaloids	−	−
2	Flavonoids	−	−
3	Saponins	−	−
4	Glycosides	++	−
5	Steroids	+++	−
6	Phenols	+++	+
7	Tannins	++	+

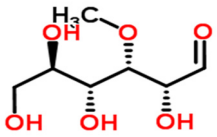
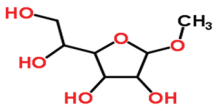
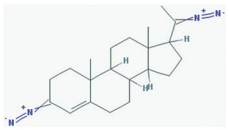
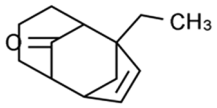
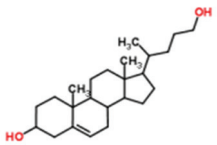
++: Highly present, +: Present, −: Absent. *H. pubescens: Holarrhena pubescens*

Table 2: Compounds identified through GC-MS analysis in *Holarrhena pubescens*

S.No	RT	Compound	Formula	Molecular weight	Peak area %	Structure	Medicinal Uses
1	2.075	1,5-Anhydroglucitol	$C_{18}H_{44}O_5Si_4$	425	6.76		Antioxidant, antidiabetic (PubChem and passonline)
2	2.249	Pentanoic acid, 2 propyl	$C_8H_{16}O_2$	144	5.46		Anticonvulsants, antimanic, antimigraine (Pubchem and passonline)
3	2.580	3-O-Methyl-d glucose	$C7H_{14}O_6$	194	1.88		Antiepileptic, anticonvulsants, anesthetic (PubChem and passonline)
4	2.675	Azetidine, 1,1' -methylenebis 2-methy	$C_9H_{19}N_2$	154	0.98		Not reported (PubChem and passonline)
5	18.350	3-Ethyl-4-methyl- -heptanol	$C_{10}H_{22}O$	158	4.03		Antiparasitic, antineoplastic, antidepressants (PubChem and passonline)
6	18.467	Methyl hexopyranoside	$C_7H_{14}O_6$	194	5.24		Anticancer, antimycotis, antiviral, antiseptic, antibiotic (PubChem and passonline)
7	18.583	Mome inositol	$C_7H_{14}O_6$	194	6.46		Antialopecic, anticirrhotic, antineuropathic, cholesterolytic, lipotropic, sweetener (PubChem and passonline)
8	18.700	1,2,3,4-CYCLOHEXANETETROL	$C_6H_{12}O_4$	148	8.03		Antiviral, antihypoxic, antitoxic, antipruritic, allergic (PubChem and passonline)
9	18.775	Alpha–D-methyl hexofuranoside	$C_7H_{14}O_6$	194	9.40		Analeptic, anti-inflammatory, anticarcinogenic, antiviral (PubChem and passonline)
10	19.504	alpha.-Methyl mannofuranoside	$C_7H_{14}O_6$	194	18.26		Antiinfective, antineoplastic, antihypoxic, antitoxic (PubChem and passonline)

(Contd...)

Table 2: *(Continued)*

S.No	RT	Compound	Formula	Molecular weight	Peak area %	Structure	Medicinal Uses
11	19.577	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	18.43		Antifungal, skin irritation, inactive, antiviral, eye irritation, inactive (PubChem and passonline)
12	21.994	BETA.-D-Mannofuranoside,	$C_7H_{14}O_6$	194	4.77		Antiinfective, lipotropic Respiratory analeptic, antihypoxic (PubChem and passonline)
13	37.724	Diazoprogesterone	$C_{21}H_{30}N_4$	338	1.69		Antipruritic, allergic, antiinfertility, female, oxytocic (PubChem and passonline)
14	37.967	5-ethyltricyclo[4.3.1.1 (2,5)] undec-3-en-10-one	$C_{13}H_{18}O$	190	7.22		Not reported (PubChem and passonline)
15	39.846	5-Cholene, 3,24-dihydroxy	$C_{24}H_{40}O2$	360	1.40		Anesthetic, antipruritic, allergic, anticarcinogenic, anti-inflammatory (PubChem and passonline)

GC-MS: Gas chromatography–mass spectrometry, *H. pubescens: Holarrhena pubescens*

methanol leaf extracts of *H. pubescens*.

DISCUSSIONS

The plant kingdom represents an enormous reservoir of biologically active compound with various chemical structures and protective/disease preventive properties. These phytochemicals often known as secondary metabolites are present in higher plants. They include alkaloids, steroids, flavonoids, saponins, glycosides, phenol, tannins, and many others. The active principles of many drugs found in plants are secondary metabolites. This work showed that *H. pubescens* petroleum ether and methanol leaf extract is rich in some phytochemicals such as flavonoids, tannins, cardiac glycoside, and saponin. These compounds have been known to possess medicinal activities particularly antibacterial activity.[8] The result of this study was not in correlation with the report of Aja *et al.*[9] which revealed the presence of alkaloids and absence of glycosides in *Talinum triangulare* leaf in both dry and wet samples. Offor *et al.*, 2015,[10] and Aja *et al.*, 2015,[11] also reported the presence of all the phytochemicals in various concentrations in *Terminalia catappa* leaf and *Cajanus cajan* leaf and seed, respectively. Arunprasath and Gomathinayagam,[12] in 2014, reported that maximum amount of all the compounds such as alkaloids, flavonoid, glycosides, steroids, phenols, tannins saponins, and resins in leaves was present in methanol extract than the petroleum ether extract.

In recent years, the interest for the study of the organic compounds found in plants and their activity has increased. The GC-MS is an ideal technique for qualitative and quantitative analysis of active components in plant.[13] The aim of the present study was to confirm the phytochemicals present in the plant extracts and to evaluate their antioxidant activity. Understanding the availability of phytochemical compounds and pharmacological properties, GC-MS is a valuable tool for reliable and novel identification of phytocompounds.[14] In the present study, 15 compounds have been identified from the methanol extract of the plant leaves of *H. pubescens* by GC-MS analysis. Thus, this type of GC-MS analyses is the first step toward understanding the nature of

Table 3: Antioxidant DPPH activity of *Holarrhena pubescens* leaf extracts in different concentration

S. No	Name of the extract	% of inhibition					Comparison of activity
		50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml	Methanol > ethanol
1	Ethanolic extract	30.01 ± 0.96	35.6 ± 0.88	38.5 ± 0.76	40.6 ± 0.88	43.6 ± 0.88	
2	Methanolic extract	57.6 ± 0.88	60.8 ± 0.94	68.8 ± 0.94	72.1 ± 0.94	75.4 ± 0.86	
3	Ascorbic acid	61.0 ± 0.93	66.3 ± 0.88	74.±0.96	78.8 ± 0.92	85.2 ± 0.92	

active principles in this medicinal plant[15] and this type of study will be helpful for further detailed study. Further investigation into the pharmacological of *H. pubescens* from various solvent extracts and detailed phytochemistry may add new knowledge to the information in the traditional medical systems. The scavenging activity of crude methanolic extract and its fractions is summarized in Table 3. Potential scavenging results are the higher percentage of inhibition which was observed in 250 µg/ml of ethanol extract followed by 250 µg/ml of methanol extract against the standard ascorbic acid (85.2 ± 0.92). The present results of *H. pubescens* leaf crude extract and its fractions indicate that they possess potential scavenging properties and scavenge the free radicals in the form of DPPH. Similar results were also reported by Ahmad *et al*.[16] and Chouhan *et al*.[17] for *Euphorbia prostrate*.[18] These indicate that seeds of *H. pubescens* are rich in glycosides, phenols, and tannins, which are responsible for antioxidant activity.

CONCLUSION

The *H. pubescens* extracts could therefore be seen as a potential source for useful drug. A total of 15 chemical constituents have been confirmed from the methanol extracts of the leaves of plant *H. pubescens* by GC-MS analysis. Extracts from *H. pubescens* showed varying antioxidant (free radical scavenging) activities when compared to Vitamin C. The results suggest that the antioxidant activity of *H. pubecens* may contribute to their claimed medicinal property. The plant has many traditional uses in anemia, colic pain, diarrhea, hematuria, menorrhagia, obstetric conditions, spermatorrhea, splenomegaly, and decoction beneficial in chronic dysentery and bleeding piles. However, very less work has been on this plant and there is further more scope of scientific investigation. In addition, these active constituents may be responsible for the medicinal characteristics of the polyherbal extract.

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REFERENCES

1. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol 2005;4:685-8.
2. Okwu DE. Flavouring properties of spices on cassava Fufu. Afr J Roots Tuber Crops 1999;3:19-21.
3. Ogukwe CE, Oguzie EE, Unaegbu C, Okolue BN. Phytochemical screening on the leaves of *Sansevieria trifasciata*. J Chem Soc Nigeria 2004;29:8-10.
4. Anonymous. Wealth of India. Vol. 5. New Delhi: PID, CSIR, Hillside Road; 1959. p. 103.
5. Gafner S, Wolfender JL, Nianga M, Hostettmann K. A naphthoquinone from *New bouldia laevis* roots. Phytochemistry 1998;48:215-6.
6. Harborne JB. Phytochemical Methods of Analysis. Vol. 64. London: Jackmann and Hall; 1973. p. 190.
7. Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol 1995;28:25-30.
8. Nweke OL, Nwachukwu N, Aja PM, Agbafor KN, Nwaka AC, Ezeilo U. Phytochemical and gas chromatograpy-mass spectrophotometeric (GC-MS) analyses of *Vitex doniana* leaf from Abakaliki, Ebonyi state. IOSR J Pharm Biol Sci 2015;10:33-8.
9. Aja PM, Okaka AN, Onu PN, Ibiam U, Urako AJ. Phytochemical composition of *Talinum triangulare* (water leaf) leaves. Pak J Nutr 2010;9:527-30.
10. Offor CE, Ugwu PC, Okechukwu PM, Aja PM, Igwenyi IO. Proximate and phytochemical analyses of *Terminalia catappa* leaves. Euro J Appl Sci 2015;7:9-11.
11. Aja PM, Alum EU, Ezeani NN, Nwali BU, Edwin N. Comparative phytochemical composition of *Cajanus cajan* Leaf and Seed. Int J Microbiol Res 2015;6:42-6.
12. Arunprasath A, Gomathinayagam M. Qualitative study of *Costus speciosus* (Koen ex. Retz.) Sm. and its potentiality against human pathogenic microbes. Int J Pharm Biol Arch 2014;5:93-8.
13. Liu RH. Health benefits of fruits and vegetables are from additive and synergic combinations of phytochemicals. Am J Clin Nutr 2003;78:517-20.
14. Kumar SS, Krishnan NR. Chromatographic fingerprint analysis of *Naringi crenulata* by HPTLC technique.

Asian Pal. J Trop Biomed 2011;1:S195-8.
15. Neeharika V, Swetha T, Humaira F, Reddy BM, Sama V. Anti-nociceptive, anti-inflammatory and antipyretic effects of *Croton* leaves. Indian Drugs 2012;49:49-12.
16. Ahmad M, Shah AS, Khan RA, Khan FU, Khan NA, Shah MS, *et al*. Antioxidant and antibacterial activity of crude Methanolic extract of *Euphorbia prostrate* collected from District Bannu (Pakistan). Afr J Pharm Pharmacol 2011;5:1175-8.
17. Chouhan AS, Mathur K, Manoj G, Yadav SK. *Holarrhena pubescens* Wall Ex. Don: A review of Ethan botanical, phytochemical and pharmacological profile. Indian J Drugs 2017;5:71-7.
18. Hagerman AE, Riedle KM, Jones GA, Sovik KN, Hartzfeld PW. High molecular weight plant poly phenolic (tannins) as Biological antioxidants. J Agric Food Chem 1998;46:1887-92.