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A STUDY ON PHYTOCHEMICALS, ANTIOXIDANT ACTIVITY AND FT-IR ANALYSIS OF *RHAPIS EXCELSA* (THUNB.) A. HENRY

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ABSTRACT

The aim of this study is to investigate the less explored popular palm *Rhapis excelsa* leaves for its phytochemical composition, anti-oxidant activity and FTIR evaluation. The aqueous, methanol and hexane extracts were used in the study. The qualitative test confirmed the presence of terpenoids, steroids, saponins, glycosides, phytosterols, resins, phenols, flavonoids and oxalic acid. The quantitative analysis of total phenolics and terpenoids in methanolic extract revealed (11 mg GAE/g) and (130 mg/g) respectively. The hydrogen peroxide and reducing power scavenging assay were used to study antioxidant activity. The FTIR analysis studies showed characteristic peak values for various functional compounds in the extracts. The present study suggests that this plant has therapeutic potential which can be used as resources of different bioactive compounds and antioxidants.

KEYWORDS: Phytochemical; phenol; terpenoid; anti-oxidant; FTIR.

ABBREVIATIONS

RM: *Rhapis excelsa* methanol extract
FTIR: Fourier Transform Infra-Red

INTRODUCTION

Trees are nature's gift. lncreasing new inventions increase new diseases. So, it is in the hands of mankind to learn the disease and drugs for their cure.[1] Hence, there is always a need to search for potential plant products to cure these diseases. Plant extracts either as pure compounds or as standardized extracts provides unlimited opportunities for new drug discoveries.[2] Arecaceae is an Angiosperm family which comprises of 200 genera and 3000 species.[3] *Rhapis excelsa* is an ornamental plant which belongs to this family. It is commonly known as lady palm or bamboo palm. The species of *Rhapis* are distributed in southern China, peninsular Thailand, northern Sumatra and India.[4] In India, it is widely grown as ornamental plant in gardens and landscapes. *Rhapis excelsa* is a bush forming palmately leaved palm, mostly grown as potted plant. It is also grown as hedges due to its bushy habit. This beautiful palm finds its usefulness in small scale industries. In China, *R.excelsa* canes are used as umbrella handles and walking sticks. The dried fibrous basal portion of the leaf stalk is scraped and used in chinese medicine. Ashes from burnt bark stimulate blood circulation and are used as a remedy for rheumatism. It is also used to stop bleeding (external application).[5] Chromatographic fractionation of the leaf parts reported the presence of four flavonoids vitexin, vicenin-2, isoorientin and orientin. The leaves were reported to have antimicrobial activity against *Staphylococcus aureus.*[6] Hexane leaf extract of *Rhapis excelsa* showed significant activity against Chikungunya virus.[7]

Since there is lack of adequate literature on the phytochemical profile and its pharmacological activity, the present study was carried out to evaluate phytochemical screening, quantitative analysis, anti-oxidant property and FTIR analysis of *R.excelsa* leaf extract.

MATERIALS AND METHODS

Plant collection and identification

Healthy leaves of *R. excelsa* were collected during October 2015 from Mahindra city, Chengalpet, Tamil Nadu. The plant sample was identified by Dr. D. Narasimhan, a taxonomist, from Madras Christian College, Chennai and Voucher specimen was maintained as herbarium.

Preparation of plant extract

The plant sample was shade dried for 2 weeks and ground to a fine powder using an electrical blender. 10 g of sample was extracted with 100 ml of each of the solvents water, methanol and hexane separately using Soxhlet apparatus and the extract was concentrated using rotary evaporator at 50°C.[8]

Phytochemical screening

Phytochemical tests were carried out for the presence of various active components using standard procedures.[9][10] [11][12]

Estimation of total phenolic content

R.excelsa leaf total phenolic content was determined by Folin-ciocalteu reagent method[13] with modification. The crude extract (1mg) was dissolved in methanol (1ml). To the sample (0.2 ml) 10% Folin-ciocalteu reagent (1.5ml) was added and incubated in dark for 5 mins. Later 5% Na_2CO_3 (1.5 ml) was added to the solution and mixed well. Finally, the test tubes were kept in the dark for 2 h. The absorbance of blue color was read at 750 nm by UV-Spectrophotometer. Total phenolic content of methanol extract was calculated as Gallic acid equivalents (mg of GAE/g of extract).

Estimation of total terpenoids

The total terpenoids was calculated by Ferguson's method.[14] The *R. excelsa* leaf powder was soaked in 20 ml alcohol (95% ethanol) for 24 h. The filtrate was extracted with petroleum ether (60°C - 80°C) and the ether extract was treated as total terpenoids. The residue obtained was dried and weighed.

Terpenoid content (%) =

$$\frac{\text{Weight of terpenoid extract (g)}}{\text{Weight of sample (2g)}} \times 100$$

Anti-oxidant activity

Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide (H_2O_2) scavenging activity was determined by the method of Ruch, *et al.*, (1989).[15] The extract (0.1 ml) of different concentrations (100-500 µg) was dissolved in 3.4 ml of 0.1M phosphate buffer (pH7.4) and mixed with 0.6 ml of 43 mM solution of H_2O_2. The reaction mixture absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without H_2O_2. The inhibition was calculated and recorded. Ascorbic acid was used as standard. The extent of H_2O_2 scavenging of the leaf extract was calculated as

H_2O_2 scavenging effect (%) = Ac-Ao/Ac ×100

Where Ac is the absorbance of control and Ao is the absorbance of leaf extract

Reducing power assay

The reducing power assay was carried out according to the method of Oyaizu (1986).[16] The methanolic extract (2.5ml) of various concentrations (100 – 500 µg / ml) was mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 mins. After incubation 2.5 ml of 10 % trichloroacetic acid (TCA) was added. The mixtures were centrifuged at 650 rpm for 10 mins. The upper layer (5ml) was mixed with 5 ml of deionised water and 1ml of 0.1% ferric chloride. The absorbance was measured at 700 nm. Increasing absorbance indicates higher reducing power. The assays were carried out in triplicates. The activity was compared with ascorbic acid standard.

FT-IR Spectroscopic analysis

Fourier Transform Infra-Red spectrophotometer (FTIR) is the powerful analytical tool for identifying the types of chemical bonds (functional groups) present in compounds. Dried methanol extract of *R.excelsa* leaf was used for FTIR analysis. 10 mg of dried extract powder was mixed with KBr salt using a mortar and pestle. Then, the mixture was compressed into a thin pellet. Infrared spectra and peak values were recorded on a Shimadzu IR (Japan), between 4000 – 400 cm^{-1}.

RESULTS

Phytochemical analysis

The phytochemical screening of *R.excelsa* leaf extract showed the presence of phenols, terpenoids, saponins, glycosoids, phytosterol, resins and oxalic acid. The results are given in the table .1

Table 1: Phytochemical analysis of *R. excelsa* leaf extract.

S.NO	PHYTOCHEMICAL CONSTITUENTS	AQUEOUS	METHANOL	HEXANE
1	Alkaloids	_	_	_
2	Carbohydrates	_	_	_
3	Terpenoids	+	+	+
4	Saponins	+	+	+
5	Steroids and Triterpenoids	+	+	_
6	Anthroquinone	_	_	_
7	Glycosides	+	+	_
8	Protein and Aminoacids	_	_	_
9	Phlobatannin	_	_	_
10	Cardiac glycosides	+	_	_
11	Gums and Mucilage	_	_	_

12	Fixed oils and Fats	_	_	_
13	Phytosterol	+	+	_
14	Anthocyanin	_	_	_
15	Resin	+	+	+
16	Fatty acids	_	_	_
17	Coumarin	_	_	_
18	Phenols	+	+	_
19	Flavonoids	+	+	_
20	Tannins	_	_	_
21	Oxalic acid	+	_	_
22	Inorganic acids	_	_	_

Key: (+) indicates presence ;(-) indicates absence

Estimation of phenolic content

Total phenolic content (TPC) of *R. excelsa* leaf extract is expressed in terms of gallic acid equivalents (standard curve equation y= 0.001+0.014, R^2 = 0.993).The TPC of methanolic extract was found to be 14.84 mg GAE/g.

Estimation of terpenoid

The total terpenoid content of *R.excelsa* leaf is represented in table.2

Table2. Total terpenoid content of *R.excelsa*

Plant extract	Amount of terpenoid (mg/g)	% of terpenoid
Ethanol	130	13%

Hydrogen scavenging activity

H_2O_2 has strong oxidizing properties. The percentage of inhibition of *R. excelsa* leaf extract at various concentrations (100-500 µg) was evaluated and compared with ascorbic acid using Ms Excel 2007. The results are given in fig.1.

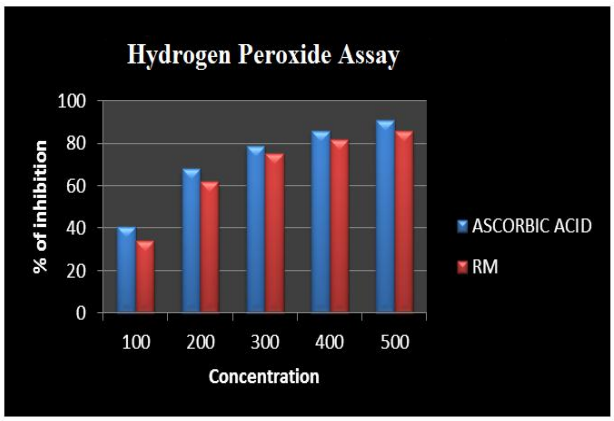

Fig.1: H_2O_2 scavenging activity showing % of inhibition of ascorbic acid and *R.excelsa* at various concentrations.

Reducing power assay

The reducing power activity of methanolic extract of *R.excelsa* was evaluated and compared with ascorbic acid. The results are given in fig.2. The reducing property of RM and ascorbic acid were found to increase with increasing concentration.

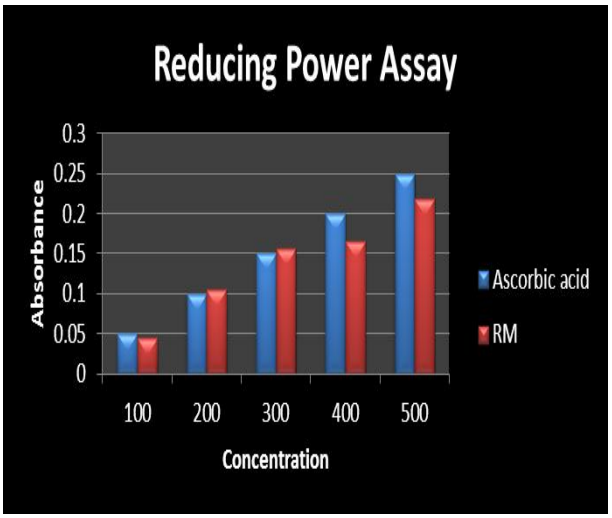

Fig 2: Reducing power activity showing absorbance of ascorbic acid and *R.excelsa*

FTIR spectral data interpretation

The FTIR spectrum of methanol leaf extract revealed the presence of various functional groups like alkenes, alkanes, nitro compounds aldehydes, ketones, amines and carboxylic acid. Thus, the result confirms the presence of active constituents like terpenoids, phenols, glycosides, saponins and steroids. The FT-IR spectrum of *R.excelsa* leaf extract was shown in fig.4.

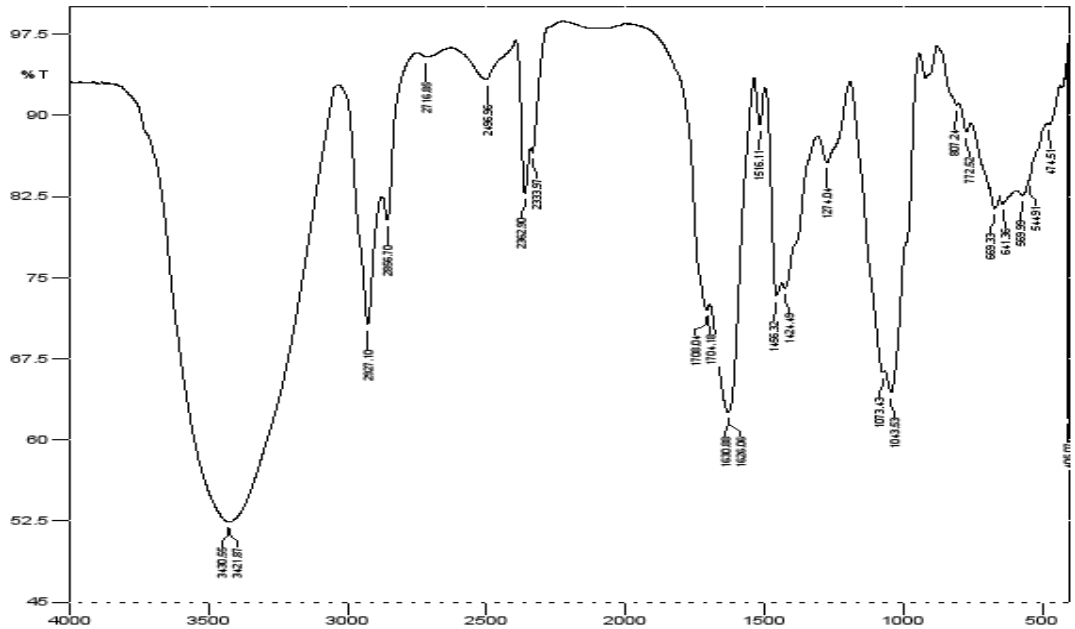

Fig .3: FTIR spectrum of *R.excelsa* leaf extract

DISCUSSION

Exploitation of rare medicinal plants with pharmacologically potential compounds always brings threat to their survival. Overexploitation of these plants may lead to their destruction. Hence, easily grown and propagated ornamental plants with potential phytocompounds provide a better solution for this problem. Reports on such ornamental plants being used as a cure in traditional systems of medicine gives us a lead for the search of potential bioactive compounds. In this study, one such easily propagated ornamental plant *Rhapis excelsa* was subjected to phytochemical screening. Phytochemicals were screened from *R.excelsa* leaf extracts in aqueous, methanol and hexane medium. The optimum results were obtained in methanol extract. So, it was used further in all the other experiments conducted in the study. Phytochemical analysis of leaf extract revealed the presence of phenols, terpenoids, glycosoids, saponins, steroids and oxalic acids. These constituents are known to exhibit many biological and therapeutic properties.[10]

The presence of phenols provides evidences for pharmacological activities like anti-oxidant, wound-healing and Anti-inflammatory action.[18] [19] Terpenes act as allelopathic agents, insect repellants and effective anti-oxidant. They are reported to act as anti-viral, anti-malarial and anti-bacterial activity.[20] [21] Steroids derived from plants are used in the treatment of rheumatoid arthritis, asthma, skin inflammation and for hormonal control. Saponins are a group of secondary metabolites and non-volatile surfactants. They have been reported to have anti-microbial, anti-tumor, anti-insect hepatoprotective, haemo-lytic and anti-inflammatory action activities.[21]

The quantitative analysis of the leaf extract showed of 11mg GAE/g of phenols and 130 mg/g of terpenoids. The presence of these active constituents (phenols and terpenoids) in *R. excelsa* leaf extract is responsible for anti-oxidant activity. The hydrogen peroxide scavenging activity was detected and compared with ascorbic acid. H_2O_2 is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. H_2O_2 can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects.[22] Reducing power assay revealed that assayed plant sample was able to reduce the ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in concentration dependent manner. The methanol leaf extract shows moderate reducing power with that of stan dard ascorbic acid.[23]

Analysis of methanol leaf sample using FT-IR technique reveals various functional groups. The result shows that the plant is medicinally important due to the presence of various chemical groups like carboxylic acids, aldehydes, alkanes, alkenes, nitro compounds, and ketones. The results obtained confirms it confirms the presence of phytochemicals like terpenoids, phenols, glycosides and saponins. The terpenoids were found to be present due to the presence of C-H stretch at 2856.7 and 2927.1 cm^{-1}. The peaks at 3421.87 and 3430.55 cm^{-1} assigned to O-H stretch shows the presence of phenols. The presence of C=O stretch at 1704.18 and 1708.04 cm^{-1} reveals the presence of saponins.[24] [25] So, the presence of these chemical group enlightens the fact that the plant is medicinally important and can be further taken for study for locating bioactive compounds to find its significance in the pharmaceutical industries.

CONCLUSION

The present study indicates that *R.excelsa* leaf is a rich source of secondary metabolites. The presence of active

constituents shows moderate to significant anti-oxidant activity. So the present study suggests that the tested material can be used as a source of anti-oxidants in pharmaceutical industry.

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