Plant Archives
Journal home page: www.plantarchives.org
DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.045

PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF ROOT EXTRACTS OF *PARKIA BIGLANDULOSA* (WIGHT&ARN.)

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(Date of Receiving-09-10-2020; Date of Acceptance-05-01-2021)

ABSTRACT

The current research work estimated to explore the qualitative and quantitative analysis of the major bioactive constituents of medicinally imperative plant *Parkia biglandulosa* in, the five solvent (petroleum ether, chloroform, ethyl acetate, methanolic and distilled water) root extracts of *Parkia biglandulosa* were predictable and characterized using GC-MS analysis. The explorations were accepted out in terms of five solvent extractions, intact extractive values, qualitative analysis, quantitative estimation and characterization of phytochemicals. The percentage value of yield extraction in petroleum ether extract was 34.74%, chloroform extract was 24.2%,ethyl acetate extract was 25%, methanol was 50% and distilled water was 54%. The preliminary phytochemical analysis showed the presence of alkaloids, flavonoids, terpenoids & phenolic compounds. The total metabolites content of five solvent extracts were compared with standards. The GC-MS analysis reveals the presence of various metabolites in three solvent(petroleum ether, ethyl acetate, methanol) extracts. It signifies that results revealed the presence of various bioactive metabolites which could be demoralized for their credible solicitations for therapeutic resolves.

Keywords: Phytochemistry, HP-LC, GC-MS, Stigma sterol, Gamma sitosterol, Alkoloids

INTRODUCTION

Parkia biglandulosa Wight &Arn. belongs to the family of Leguminosae and the sub family Mimosoideae. This tree is distributed in tropical regions of South America, Asia, Africa and India (Hopkins 1983, Hall *et al*.,1997). Frequently, In India it is found in parks, ornamental tree in gardens and alsoas avenue tree on roadsides (Pingale *et al*., 2016)". This plant belongs to '*Parkia'* genus and the tribe "Parkieae". It's flowering and fruiting occurs generally in the month January – March. Hence its native name is also referred to as "Chenduphul". The entire inflorescence of this plant has a very soft arrival, once the flower is dry and the core is pretty hard (Trease and Evans 2016).

Phytochemical metabolites are certainly found in the therapeutic plant parts like root, leaves, bark, vegetables and fruits that have express mechanism and protect from innumerable diseases. Phytochemical studies have extended a lot of interest among the researchers due to the amplification of newer technology and advanced outcome. These practices play a substantial role in finding of important material for pharmaceutical industry (Alston and Turner,1963). Plants have constituents that persuade a great interest due to their multipurpose applications (Baris *et al*., 2006). It is predictable that 14-18% of higher plant are used remedially and related to 74% of pharmacologically vigorous plant are revealed after following up on ethnomedicinal practice of the plants (Ncube *et al*., 2008). Plants are able with several phytochemical compounds such as alkaloids, terpenoids, flavonoids, phenolic, lignins, stilbenes, tannins, quinones, coumarins, vitamins, amines, betalains, and other constituents which are rich cause of free radical scavengers (Chaithanya *et al*.,2018,Gracelin *et al*., 2012). They are also antioxidant compounds which possess anticarcinogenic,antimutagenic,antibacterial and antiviral activities (Ncube *et al*., 2008, Zheng and Wang 2001). Additionally, assimilation of expected antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with mature (Sala *et al*., 2002). Hence, with this preview, the current work has carried out to evaluate the Phytochemical Screening and Characterization of root extracts from *Parkia biglandulosa.*

MATERIALS AND METHODS

Sample collection: The roots were collected from the Botanical garden, Department of Botany and Tennis court area, University Science College Campus, Tumkur University, Tumakuru.

Preparation of extract: Healthy root samples of *Parkia biglandulosa* plant were collected. Then washing with tap water two to three times and then with distilled water .These rootsamples will be shade dried and stored in refrigerator at 4^0C until use. The dried root samples were powdered by grinding in (mixer grinder) and 100g of each powder were extracted in a Soxhlet apparatus with 1000ml of different solvents (petroleum ether, chloroform, ethyl acetate, methonol and distilled water) for 24 hrs. The extracts were evaporated in rotary evaporator to obtain the powdered extracts which will be tested for further studies. Percentage yield of the plant extract was calculated rendering to the formul

Percentageyield of the extract $=X_a/X_b$ X100

Where =X_a plant material weight after extraction process

X_b= Plant material weight taken before extraction

Preliminarily Phytochemical Screening (Qualitative analysis): The crude extracts viz., Petroleum ether, Chloroform, Ethyl acetate, Mathonol and Distilled water extracts of P. biglandulosa obtained from each of the solvent were subjected for the following qualitative tests were done for the presence of various metabolites as follows.

Test for Alkaloids

Mayer's test: A few drops of the Mayer's reagent was treated with 0.5 ml 0f extracts. Appearance of white or pale yellow precipitate indicates the presence of alkaloids.

Hager's test: 0.5 ml of extracts was taken in a test tube, a few drops of hager's reagent was added. Formation of yellow precipitate indicated the presence of alkaloids.

Wagners test: 0.5 ml extracts was acidified with hydrochloric acid and a few drops of wagners reagent were added. Appearance of yellow or brown precipitate indicates the presence of alkaloids.

Test for Flavonoids

Lead acetate test: 0.5 ml of extract was taken and few drops of 10% lead acetate solution were added. Appearance of yellow color precipitate indicates the presence of flavonoids.

Test for Phenols

Lead acetate test: 0.5ml of extract was taken and 0.5 ml of 1% lead acetate solution was added and the appearance of precipitate indicates the presence of tannins and phenolic compounds.

Ferric chloride test: 0.5ml of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black color indicates the presence of tannins.

Sodium hydroxide test: 0.5ml of extract was dissolved in 0.5 ml of 20% sulphuric acid solution. Followed by addition of few drops of aqueous sodium hydroxide solution, it turns blue which indicates the presence of phenols.

Test for Terpenes.

Salkowski test:A few drops of concentrated sulphuric acid was added to the 0.5ml of extracts, shaken and allowed to stand, lower layer turns yellow indicating the presence of terpenoids.

Quantitative analysis of root extracts. Estimation of above phytochemicals from root extracts by using HPLC.

HPLC analysis of (Alkoloids, Phenols, Flavonoids and Terpenoids): The analysis was made (Waters model no. 486; Waters Corp., Milford, MA, USA) on C18 column (symmetry, 4·6mm×250mm) in isocratic mode with the mobile phase methanol and water in the ratio 7:3 with the RP-HPLC C-18 column at a flow rate of 1mL/min. The standards (Caffiec acid, Gallic acid, Rutin, Tannic acid) with the concentration 0.1mg/mL and sample (1mg/mL) were dissolved in mobile phase and 20µL was injected and the elution was monitored at(230nm, 254nm,272nm, 210nm)(Ashok kumar *et al*.,2008). The amount of different compounds present in the sample was estimated using the formula,

$$\frac{\text{Area of Sample}}{\text{Area of Standard}} \times \frac{\text{amount of standard}}{\text{standard}} \times \frac{\text{dilution}}{\text{dilution}} \times \text{Mean weight}$$

sample amount

Characterization by GC-MS analysis: The root extracts obtained was subjected to gas chromatography and mass spectroscopy for the determination of bioactive volatile compounds.

GC-MS analysis of the three solvent extracts(Petroleum ether, Ethyl acetate and Methanol)of root of *Parkia biglandulosa* was performed using a Schimadzu GCMS-QP2010 instrument.

The oven temperature is maintained at 220ºC at a rate of 6ºC/min; the carrier gas with a flow rate of 1 ml/min. The fragmented sampling technique was used to inject the sample in the ratio of 1:10. Retention catalogues of the compounds were determined by comparing the retention times of a series and identification of each component was confirmed by comparison of its retention index with data in the literature. Elucidation of Mass-Spectrum was carried out by using the database of National institute Standard and Technology having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components which was stored in the NIST library. The molecular formula, compound name, chemical structure and molecular formula of the components of the test materials were determined. The peak in GCMS of three solvent extract of root of *Parkia biglandulosa* showed the presence of the various secondary phytochemical compounds like alkoloids, flavonoids, phenols and terpenes (Rukshana *et al*., 2017).

RESULTS AND DISCUSSION

Preparation of extract: Healthy Root sample of *Parkia biglandulosa* plant was collected in the month of March 2020. The root sample were shade dried and extracted in Soxhlet apparatus. The dried samples were powdered by grinding in (mixer grinder). Soxhlet extracted samples was collected. This extraction was further used for phytochemical screening, characterization and biological activities.

Percentage yields of root extracts of *Parkia biglandulosa* using different solvents. The yield percentage may be due to various factors as yield percentage depends on type of solvents with varying polarities, extraction time and temperature, sample to solvent ratio as well as on the chemical composition and physical characteristics of the samples. The physical status and percentage yield of the

Table 1: Physical status and percentage yield of the root extracts.

Sl no	Root sample (extracts)	Quantity used for extraction		Nature of the extract	Obtained in gm	% in yield
		Powder(gm)	Solvent(ml)			
1	Petroleum ether	100	1000	Light yellow	5.6	5.6
2	Chloroform	100	1000	Brownish yellow	10.2	10.2
3	Ethyl acetate	100	1000	Maroon	25	25
4	Methanol	100	1000	Brownish	50	50
5	Distilled water	100	1000	Dark brown	54	54

Table 2:-Qualitative phytochemical screening of root extracts of *Parkia biglandulosa*.

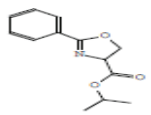
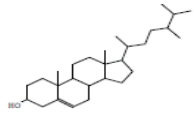
Sl.no	Tests	Root extracts				
		PE	CH	EA	ME	DW
1	Test for Alkaloids	+	+	+	++	+
2	Test for Flavonoids	+	+	+	+++	++
3	Test for Phenols	+	+	++	+++	++
4	Test for Terpenes	+	++	+++	++	+++

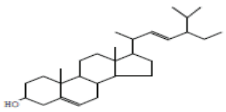
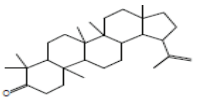
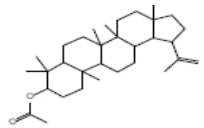
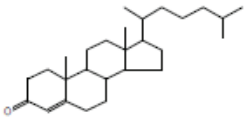
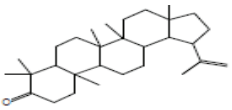
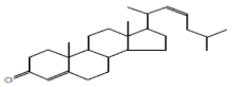
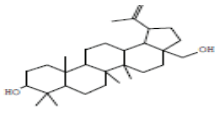
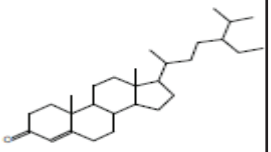
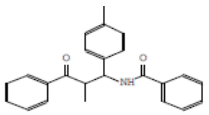
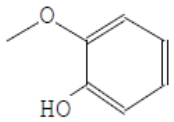
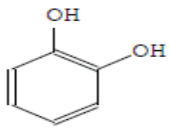
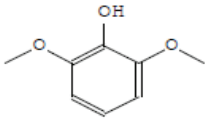
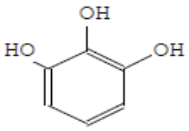
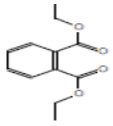
+-Presence, ++-Moderate, +++ - More

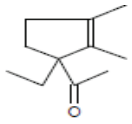
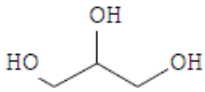
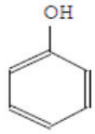
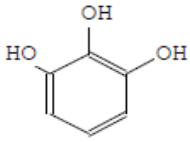
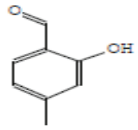
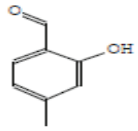
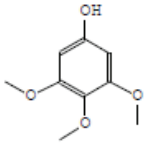
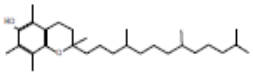
Table 3: Quantitative analysis of root extracts of various metabolites

Root Extracts	Amount in mg/ g of sample			
	Alkoloids	Flavonoids	Phenols	Terpenes
Petroleum ether	0.32	4.29	2.13	83.37
Chloroform	1.0	49.24	8.5	189.2
Ethyl acetate	8.52	81.9	27.69	833.3
Methanol	**65.3**	**317.2**	**118.30**	**135.1**
Distilled Water	15.63	133.5	263.016	571.7

Table 4 : GC-MS analysis of major compounds present in the root extracts of *Parkia biglandulosa*

Peak PE	Extracts	Retention time	Peak area %	Compound name	Molecular formula	Molecular weight	Chemical structure
1	RPE	27.500	1.20	4-oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester	C13H15NO3	233	
2	RPE	28.166	1.11	Heneicosane	C21H44	296	
3	RPE	29.646	1.18	Dotricontane	C32H66	450	
4	RPE	44.662	2.31	Ergost-5-en-3-ol,(3. beta.)-	C28H48O	400	

5	RPE	45.409	3.07	Stigmasterol	C29H48O	412	
6	RPE	46.800	8.76	Lup-20(29)-en-3-one	C30H48O	424	
7	RPE	46.941	11.02	Gamma-sitosterol	C29H50O	414	
8	RPE	47.464	14.27	Lup-20(29)-en-3-ol,acetate,(3.beta)-	C32H52O2	468	
9	RPE	48.174	2.84	Cholest-4-en-3-one	C30H48O	424	
10	RPE	48.623	6.37	Lup-20(29)-en-3-one	C27H42O	382	
11	RPE	49.096	1.95	4,22-cholestadien-3-one	C29H46O	410	
12	RPE	49.308	11.14	Betulin	C30H50O2	442	
13	RPE	51.067	13.16	Stigma-4-en-3-one	C29H48O	412	
14	RPE	51.683	1.01	2-Azapentane-1,5-dione, 4-methylene	C24H23NO2	357	
15	RME	8.781	1.73	Phenol, 2-methoxy-	C7H8O2	124	
16	RME	11.285	13.62	1,2-Benzenediol	C6H6O2	110	
17	RME	14.710	1.91	Phenol, 2,6-dimethoxy-	C8H10O3	154	
18	RME	15.235	66.08	1,2,3-Benzenetriol	C6H6O3	126	
19	RME	19.692	3.43	Diethyl Phthalate	C12H14O4	222	

20	RME	27.775	1.10	1-(1-Ethyl-2,3-dimethyl-cyclopent-2-enyl)-ethanone	C11H18O	166	
21	REA	7.573	2.13	Glycerin	C3H8O3	92	
22	REA	8.758	2.17	Phenol	C6H6O	94	
23	REA	15.283	47.09	1,2,3-Benzenetriol	C6H6O3	126	
24	REA	16.608	1.05	Benzaldehyde, 2-hydroxy-4-methyl-	C8H8O2	136	
25	REA	19.688	7.73	Benzenetriol	C6H6O3	126	
26	REA	19.964	2.41	Phenol, 3,4,5-trimethoxy-	C9H12O4	184	
27	REA	42.521	0.07	Vitamin E	C29H50O2	430	

(RPE- Root Petroleum Ether, REA- Root Ethyl Acetate, RME- Root methanol extracts).

extracts as specified in the (Table 1).

Qualitative phytochemical analysis: Phytochemical screening of root extraction was used for preliminary phytochemical analyses. The following qualitative tests were done for the presence of various metabolites as shown in this (Table 2).

Quantitative analysis of root extracts: The amount of metabolites present in the root extracts were done as shown in this Table **3.**and HPLC chromatogram for the Estimation of metabolitesfrom root Extracts given in image 3.

GC -MS Analysis of root extracts: GC-MS is the utmost methods to identify the metabolites of explosive substance, long chain, Branched chain hydrocarbons, alcohols acids, esters, etc. Peak area%, retention time, molecular formula and chemical structure were used for the confirmation of phytochemical compounds. The active values with their Retention time, Molecular formula, Molecular weight and percentage peak area are presented (Rukshana *et al*., 2017). GC/MS analysis of three solvent (Petroleum ether, ethyl acetate and methanolic extract of *Parkia biglandulosa* roote xposed the presence of various compounds (phytochemical metabolites) were revealed the remedial quality of the plant (Table 4).

Discussion: The contemporary study revealed that Percentage yield of all solvent extracts of root of *Parkia biglandulosa*. This shows high extract obtained in Ethyl acetate, Methanol and Distilled water extract. Then the Qualitative phytochemical screening exposed that it is plentiful in metabolites such as alkaloids, flavonoids, terpenoids, phenols exclusively it was found in high amount in methanol extract than other extracts (Table 1).HPLC analysis of Quantitative estimation showed that polar solvent (ethyl acetate, methanolic and distilled water) extracts contains more amount comparing to other twoNon-polar solvent (Petroleum ether, Chloroform) extracts(Table 3). Finally, characterization of GC-MS analysis of petroleum ether shows the presence of 49 compounds, ethyl acetate have 47 and methanol have 60

Table 5: Pharmacological Properties of Phytoconstituents

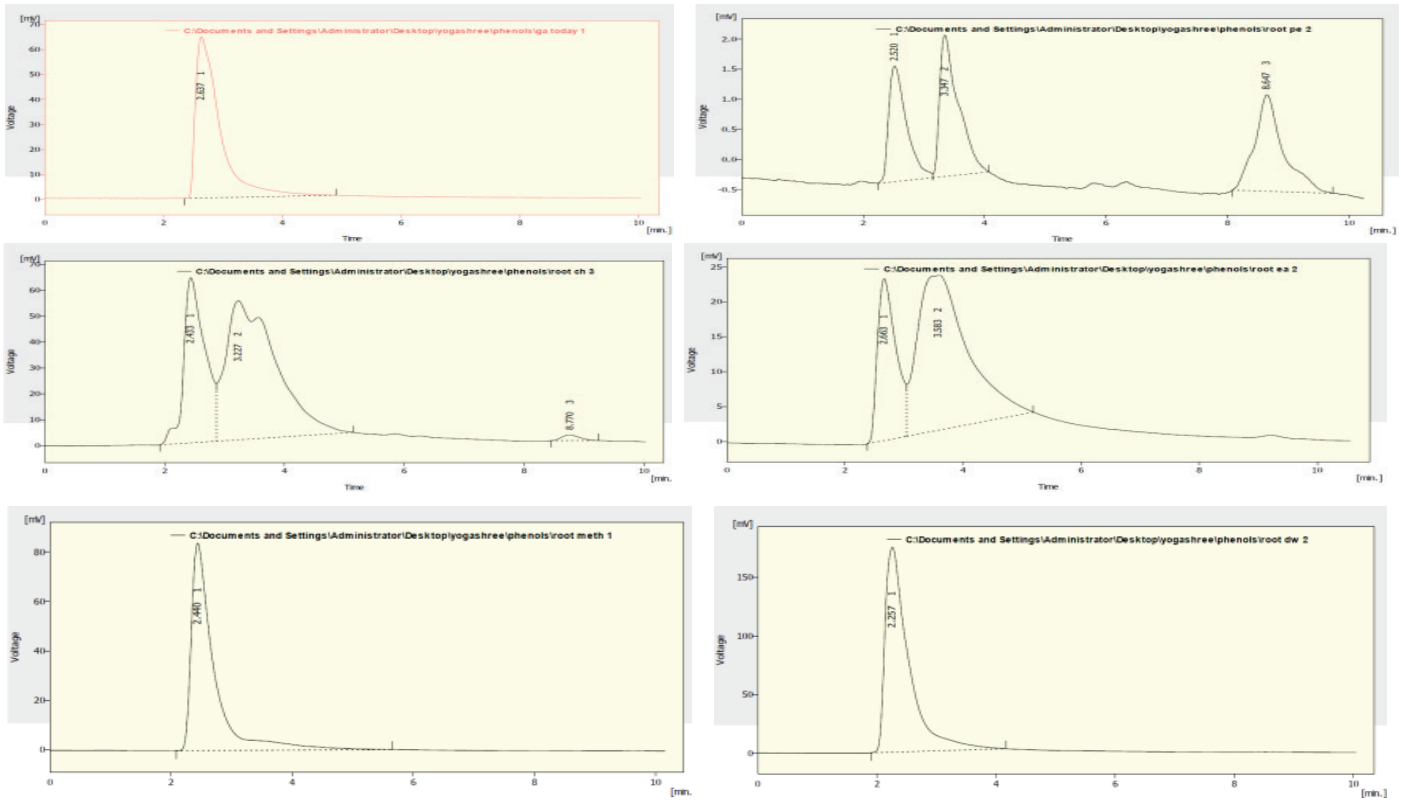
Sl.no	Compounds	Pharmacological activities
1	Phenol	Antioxidant activity (Rice *et al*., 1997).
2	1,2-benzenediol	Anti-microbial activities,anti-oxidant, anti-cancer and anti-pesticides.(Choudhary *et al*., 2019).
3	Dotricontane	Anti-microbial, antioxidant andantispasmodial activities. (Soosairaj and dons 2016).
4	Vitamin E	Antioxidant, functioning immune system and protect eyesight
5	Stegmasterol	Anti-inflammatory, Anti-osteoarthritic, antiperoxidative, thyroid inhibitory, antihepatotoxic, , antiviral, cancer- preventive. (Choudhary *et al*., 2019).
6	Heneicosane	Antimicrobial activity (Naeim *et al.,*2020).
7	Gammasitosterol	Anti-angiogenic , anticancer, antimicrobial, anti-inflammatory, anti-diarrheal and antiviral activities. Antihyperglycemic activity, hepatoprotective and antidiabetic drug (Choudhary *et al*., 2019).
8	Betulin	Antiseptic, anti-inflammatory, antiviral properties, antibacterial, antifungal and antitumour activities (Haque *et al.,*2014)
9	Diethyl phthalate	Antimicrobial activity, carcinogenic, teratogenic, hepatotoxic and endocrine effects. (Premjanu and Jaynthy 2014)
10	Glycerin	Constipation, improving hydration and performance in athletes and for certain skin conditions.Antiinflammatory (Hussein *et al.,*2017)
11	1,2,3- benzenetriol	Antibacterial and antioxidant activities. (Saeida *et al.,* 2014)
12	Ergost-5-en-3-ol,(3.beta.)-	Anti-cancerous, hypocholesterolemic, Antioxidant (Choudhary *et al*.,2019).

Plant image, B. Root image, C. Root powder image

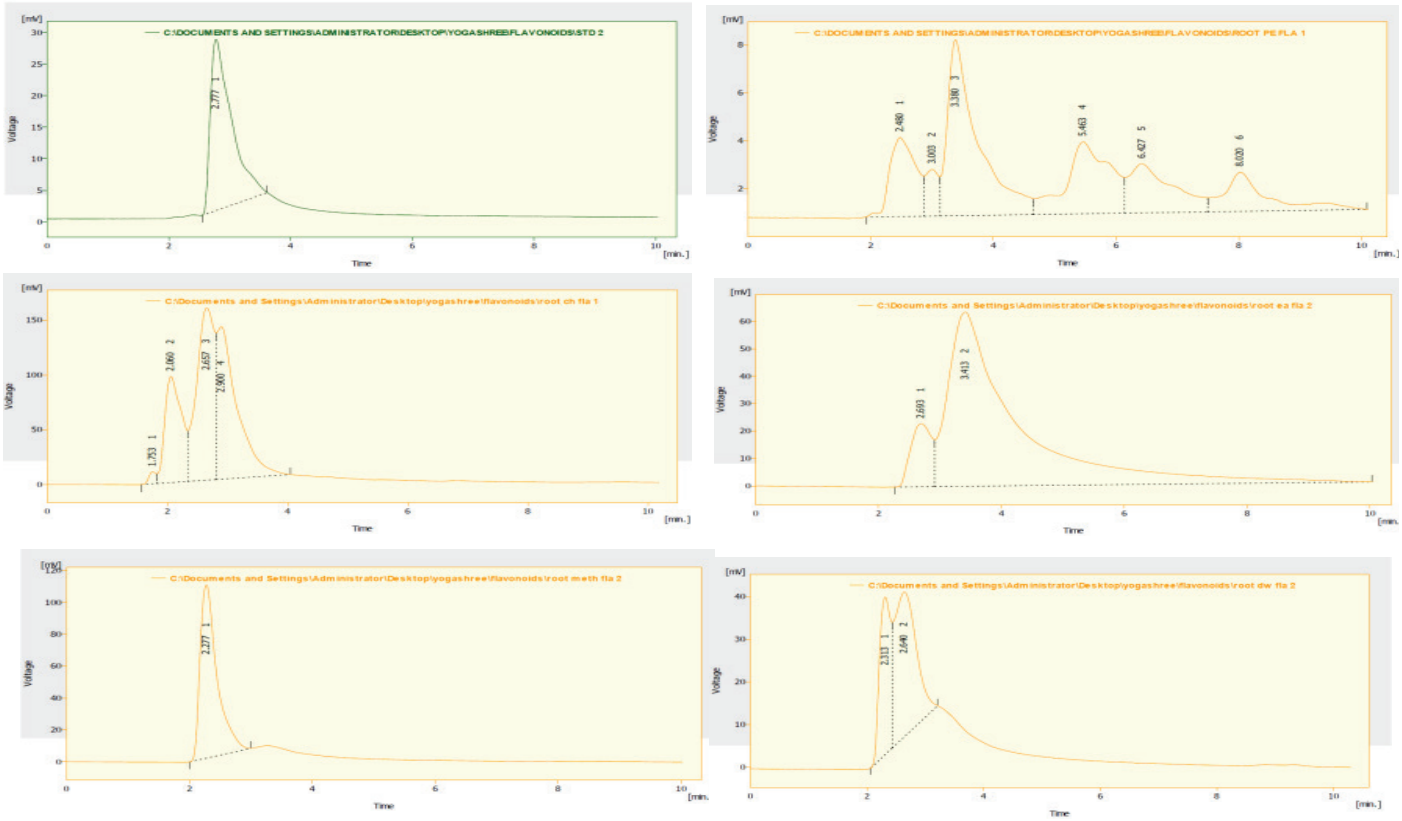
Image1: Plant and root image of *Parkia biglandulosa*.

compounds from root extracts of *Parkia biglandulosa*. The root extracts of *Parkia biglandulosa* contains active phytochemical compounds showed in the above (table 5) and these compounds play a vital role in the pharmacology field.

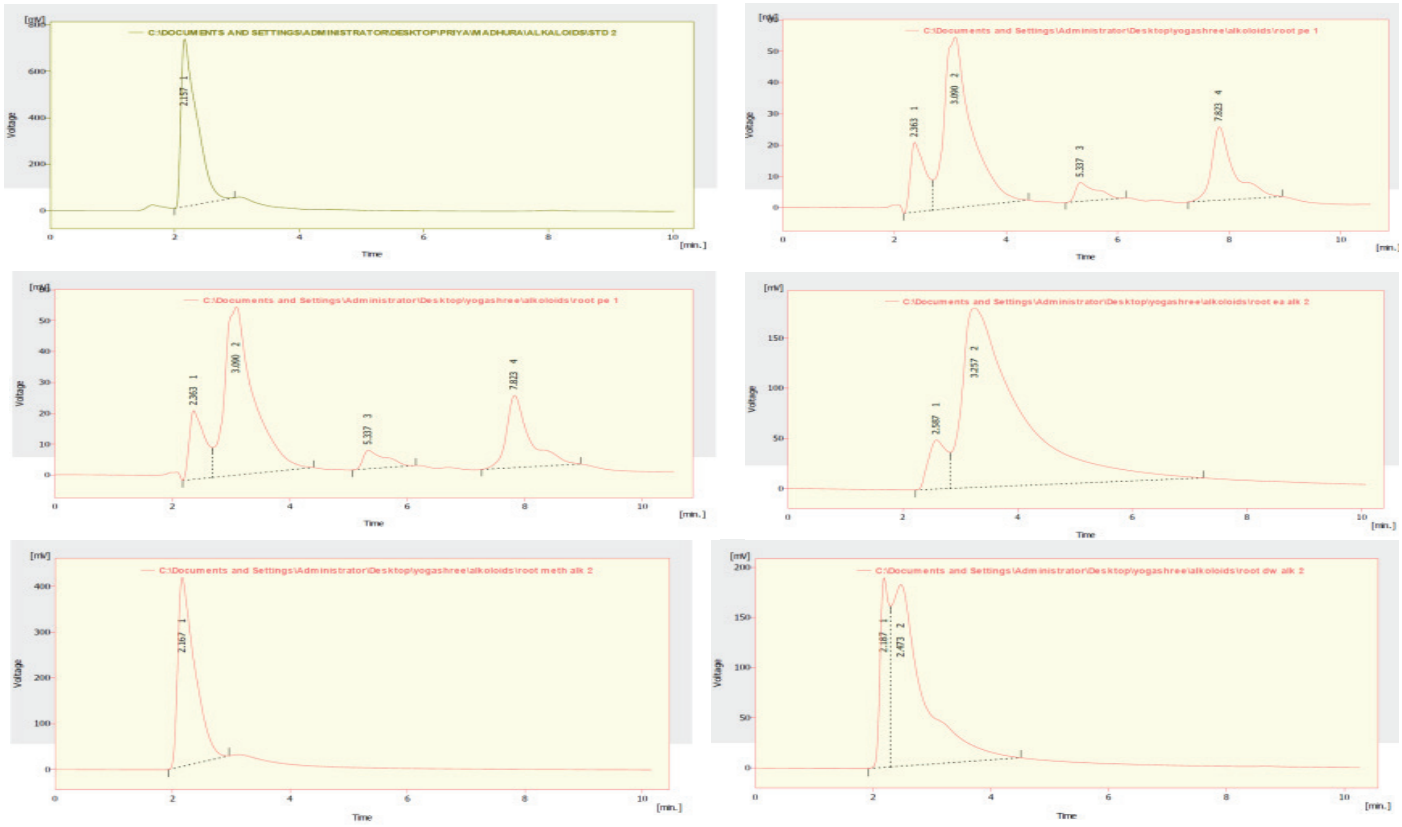
Initially, Rice *et al*.,(1997) phenolic compound shows antioxidant activity. Further, choudhary *et al*., (2019) described the presence of 1,2 benzenediol, stigma sterol, gamma sitosterol and Ergost-5-en-3-ol,(3.beta.)- Constituents extract from aerial part and callus extract of *D. glaucum*.Soosairaj and dons (2016) stated dotricontane acts as Anti-microbial, antioxidant and antispasmodial activities from Ethanolic extract of *Justicia tranquebariensis.* Then, Naeim *et al.,*(2020) in this work phytocompound heniecosane reported that Antibacterial activity of *Centaurea pumilio* L. root and aerial part extracts against some multidrug resistant bacteria. Haque *et al.,* 2014 reported that betulin is a unsurprisingly occurring triterpene, which is found in extensive amounts from the outer bark of birch trees which acts as Antiseptic, anti-inflammatory, antiviral properties,



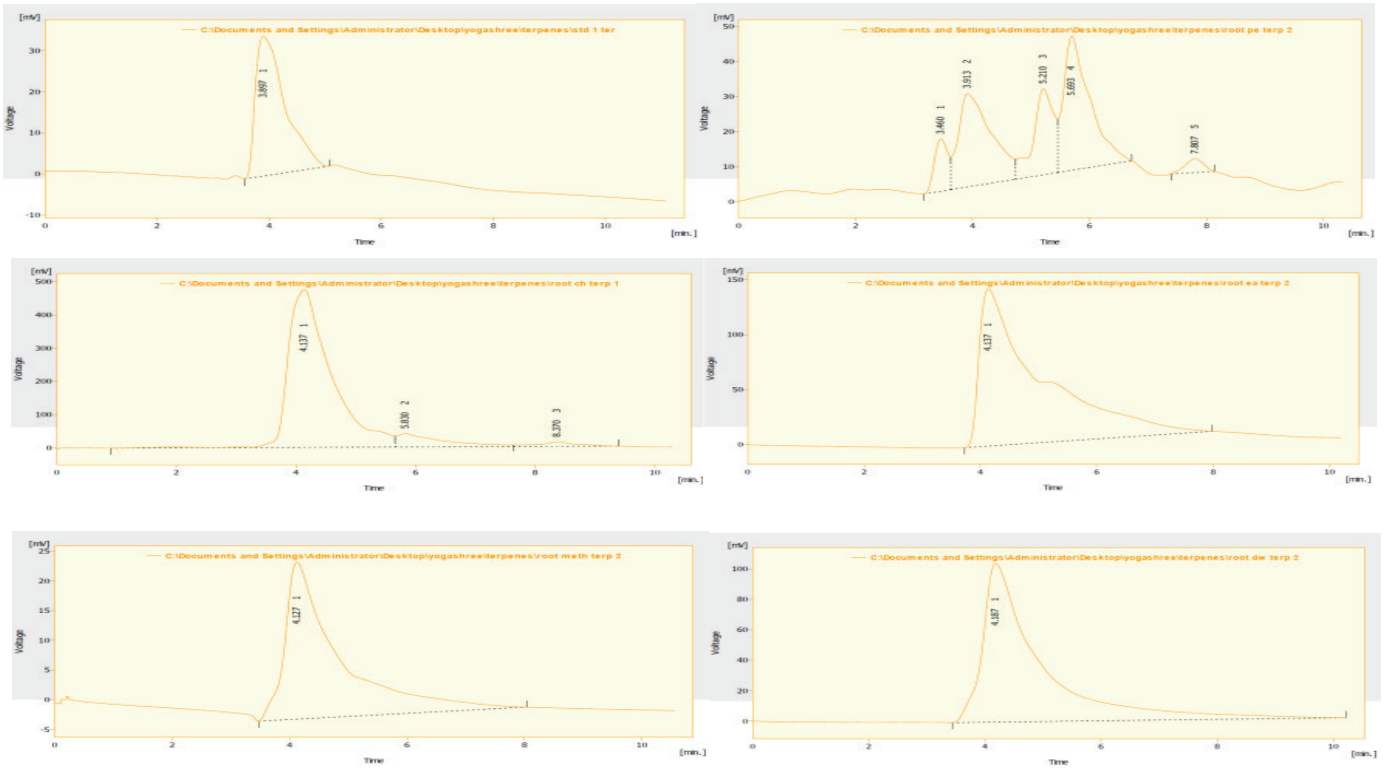
HPLC chromatogram for the Estimation of phenols from Root Extracts



HPLC chromatogram for the Estimation offlavonoids from Root Extracts



HPLC Chromatogram for the Estimation of Alkoloids from Root



HPLC chromatogram for the Estimation of Terpenes from Root

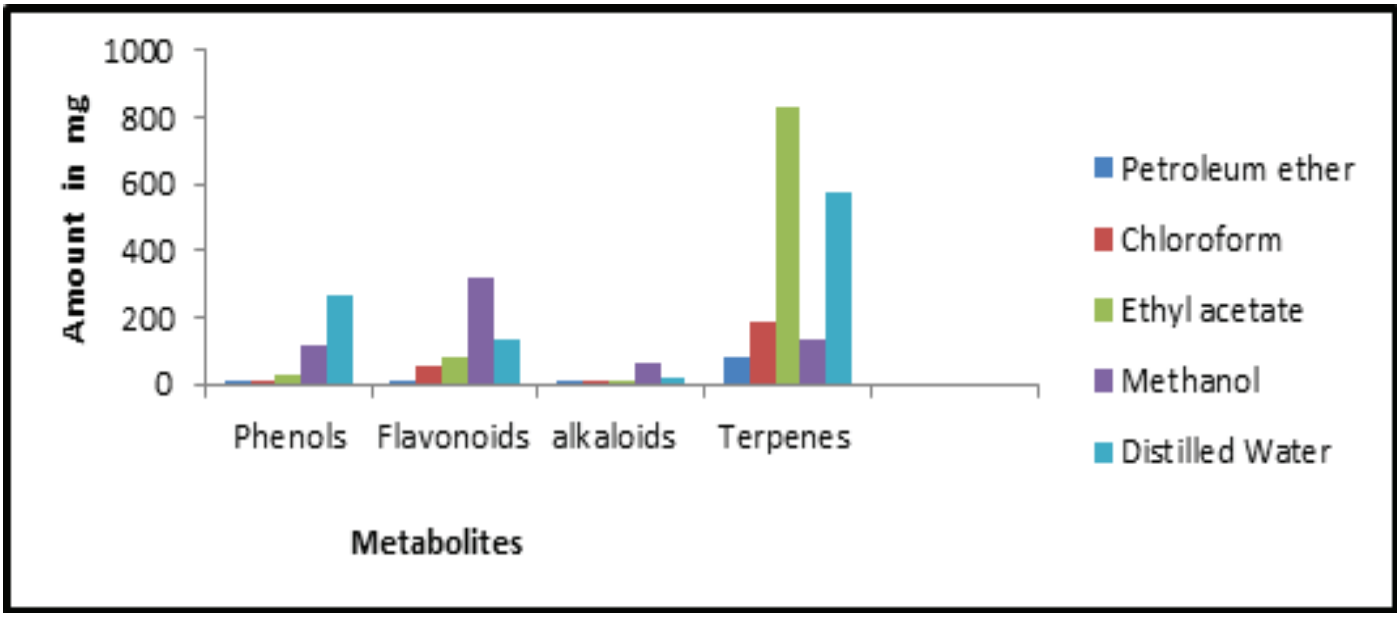


Image2 : Quantitative analysis of root extracts of different solvents.

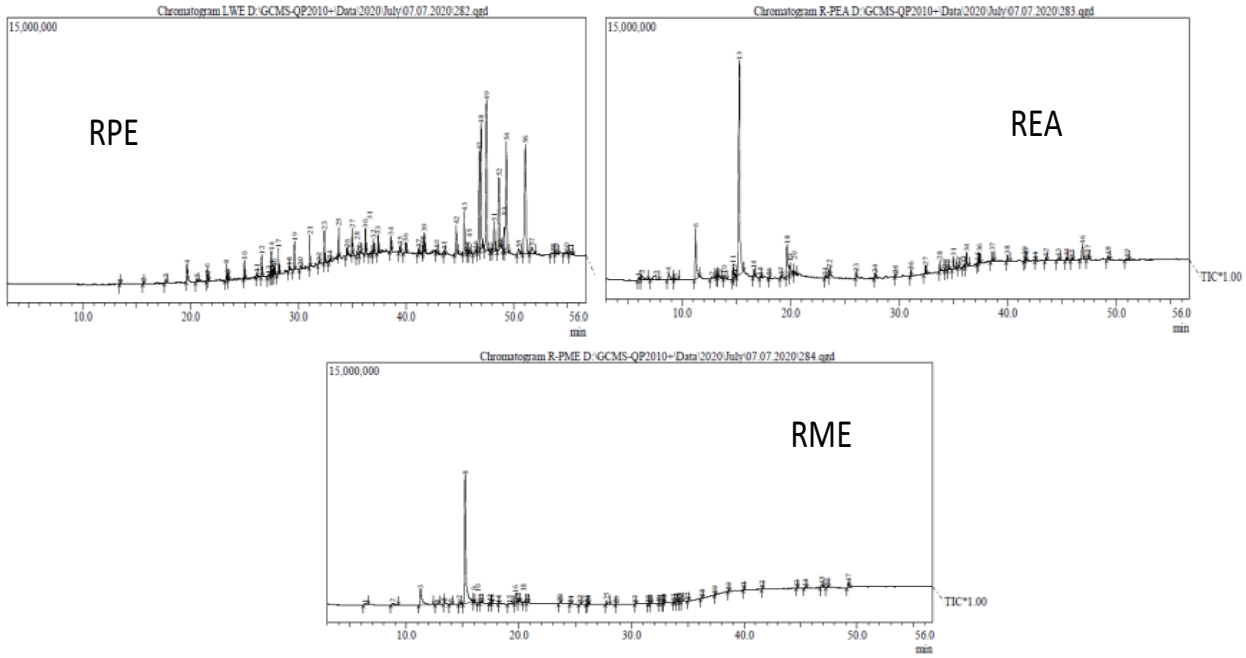


Image 3: GC- MS spectral chromatogram of root

antibacterial, antifungal and antitumour activities. Premjanu and Jaynthy (2014) described Antimicrobial activity of diethyl phthalate isolated from *Lannea coramendalica.* Additionally, Hussein *et al.,*(2017) in his work glycerin is a bioactve compound present in Leaves methanol extract of *Lepidium sativum*. Finally, Saeida *et al.,* 2014 in his report1,2,3 benzenetriol is a major compound acting an significant role towards the antimicrobial activity.

The work is in progress to find out pharmacological profile in the field of traditional medicine.

CONCLUSION

The present study conclusively demonstrate that *Parkia biglandulosa* is a good source of various phytochemicals like alkaloids, flavonoids, phenols and terpenoids, Medical plants are the potent source of human health due to its active constituents that is responsible for its numerous pharmacological activities. *Parkia biglandulosa* is a one such traditional medicinal plant which was investigated and showed that the phytochemical constituents and bioactive compounds possess the medicinal properties which makes them to be a potential species for commercial exploitation. Thus, the present work focused on the advantage of exploiting medicinal plants which are the potent source of human health, due to their presence of active compounds which are responsible for its various pharmacological activities.

Thus in our study ,*Parkia biglandulosa* a traditional folkloric plant is being investigated for presence of various bioactive metabolites that possess the remedial properties

which makes them to be potential species. It also has widespread use with extraordinary medicinal, potential which should be better explored to find new biological properties which may increase its importance as efficient medicinal plant in biodiversity.

ACKNOWLEDGEMENT

Authors are thankful to the Chairman, Department of Botany, Kuvempu University, Shankaraghatta, Shivamogga and Azyme bioscience laboratory for providing the laboratory facilities.

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