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In vitro assessment of antimicrobial, antioxidant and antiinflammatory activities from root extracts of *Parkia biglandulosa* (Wight & Arn.)

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Abstract
Background: *Parkia biglandulosa* is one such plant and its species like, *Parkia biglobosa*, *Parkia bicolor* and *Parkia roxburghii* are used conventionally as nutrition, therapeutic agents and mainly for profitable significance in many countries, which have been considered to some level, but so far away, in the literature there are very few reports on *Parkia biglandulosa*.
Method: For this preview, the main objective of this investigation is to estimate antimicrobial, antioxidant and antiinflammatory activity of *Parkia biglandulosa* root extracts by invitro method. Five different solvents (PE, CHE, EAE, ME and DW) extracts used to evaluate anti-oxidant activity, three solvent (petroleum ether, ethyl acetate and methanol) extracts are used for the analysis of anti-microbial (anti-bacterial and fungal) activity and the anti-inflammatory (protein denaturation and membrane stabilisation) activities of *Parkia biglandulosa.*
Result: Results obtained from this study the anti-microbial activity revealed that EAE shows good inhibitory effect compared with the standard against both bacterial and fungal strains. further, in antioxidant activity results shows ME and EAE has good anti-oxidant property with the IC50 values both in DPPH and ABTS assay and PE and ME has shown significant effect in anti-inflammatory activity.
Conclusion: Finally, *Parkia biglandulosa* contains excellent antimicrobial, antioxidant and antiinflammatory agents to heal several disorders.

Keywords: anti-microbial activity, Ciprofloxacin, DPPH, ABTS, haemolysis

Introduction
The plant medicines turns as the illustrative of the maximum significant arenas of traditional remedy in all over the world. Thus, the therapeutic plants are necessary to sponsor the appropriate use of herbalprescription in order to regulate their possible as a source for new [1]. Remedial plants are used for the treatment of disease since prehistoric times. So, natural medicinal products are conventionally applied for action of diseases and their vigorous ingredients have been studied for elucidation of their mechanisms of action [2]. Consequently, the product obtained naturally ensuing from plant offer a new source of biological actions that make a great impression on transmittable disease and global human healthiness [3, 4].
As an outcome, drugs derived from plants naturally by products was used to benefit manhood endure its healthiness since the dawn of prescription. Pharmaceutical findings over the past century, the phytoconstituents in plants are a essential channels. The plant active ingredients importance in agriculture and remedy of these substances has inspired significant scientific interest in the biological activities. Even though these studies, a limited range of plant species has qualified complete systematic valuation and accurate information is relatively inadequate regarding their possible part in environment. From this time, the accomplishment of a practical observation of expected products demands complete inquiries of these plants on the biological activities and their vital phytoconstituents. Plants with a long history of use in ethno medicine are a rich source of active phytoconstituents in the pharmaceutical landscape, that offer remedial or health assistances counter to numerous disorders and diseases [5]. The stabilizing operational of voluminous herbal species and herbs advises the occurrence of anti-microbial and anti-oxidant ingredients in their materials. Several therapeutic plants comprise huge quantities of antioxidants [6] [7]. For the above background this study was aimed to evaluating the anti-microbial, antioxidant and anti-inflammatory potential of five solvent extracts of root of *Parkia biglandulosa.*

Methodology
Anti-microbial studies
Antibacterial studies
The Root extracts such as PE, EAE and ME were evaluated for their anti-bacterial activity against two Gram +ve and two Gram -ve bacterial strains i.e *E.coli* [MTCC-1599], *S.typhi*.[MTCC-734], *S. aureus* [MTCC-4734] and P. syringae [MTCC- 1604] using Agar Well Diffusion Method. The DMSO were used to dissolve the extracts and the dilutions were arranged in different concentrations(500µg/ml, 250µg/ml and 125 µg/ml). They remained separated by compelling ciprofloxacin as +ve control and DMSO serve for as –ve control. The test bacterium were inoculated as a standardized suspension and incubate it for 16-18 hours at 37^0C to obtain fresh cultures. Then the sterilized petri plates were transferred to 20ml of LB agar media, new inoculum 100 µl was smeared and tolerable to dehydrated for 10mins. By using an agar punch Suitable wells are prepared on these agar plates and along with control standards diverse absorptions of each extract

were added to each of the labeled wells. The petri dishes were allowed to settle for one hour at room temperature and incubated at 37^0C for 16-18 hrs. Each extract was analyzed the antibacterial activity by calculating the distance of the zone of inhibition and the strength for each extract was interrelated with Ciprofloxacin as a standard.

Antifungal Studies

The 3 root extracts were evaluated for their anti-fungal activity compared to four fungal strains like *Aspergillus terrus, Penicillium brocae, Aspergillus flavus and Curvularia* these are categorized by laboratory, by agar well diffusion method. All the extracts were dissolved in DMSO and the dilutions were arranged in different concentration (500 µg/ml, 250 µg/ml and 125µg/ml). they separated by using fluconazole used as +ve control and DMSO served as -ve control. The fungal strains of standardized suspension were inoculated and incubated for 3-4 days at room temperature to obtain fresh culture. Then 20 ml of PDA agar media were poured to sterilized petri dishes, fresh inoculum 100 µl was smeared and allowed it to dry for 10mins. In the agar plates using an agar punch suitable wells were prepared and along with standards dissimilar concentrations of each extract used as a control remained added to all the labeled wells. each Petri dishes incubated for 3 to 4 days in room temperature. The anti-fungal activity of root extracts was analyzed by calculating the diameter of the inhibition zone and the potency of each compound was correlated with Fluconazole.

***In vitro* Anti-Oxidant Activity**

The anti-oxidant properties of the root extracts were determined with reference to ABTS and DPPH assay method.

Determination of Free Radical Scavenging Activity by ABTS

The total anti-oxidant activities of the root extracts were dignified by ABTS essential cation staining assay. This was formed by answering 7mM of ABTS in distilled water with 2.4mM Potassium per sulphate and kept it in the dark chamber for 12-16 hr. at room temperature. Previous to assay, the solution was diluted with methanol and equilibrated to give absorbance at 734 nm. The Root extracts of different dilutions to this 3ml of diluted ABTS solution was added. After 30 min of incubation the absorbance was measured at room temperature. without sample ABTS Reagent was used as control. Against Gallic acid Percentage of ABTS radical inhibition was plotted and IC 50 values were resolute. The % of inhibition of all extracts was calculated as

Inhibition % = [absorbance (blank) – absorbance (sample)/absorbance (blank)] x100

Determination of DPPH- Free Radical Scavenging Assay DPPH

scavenging assay was predictable by accumulation of diverse concentration of root extracts to 0.1 mM of (1-1 - diphenyl - 2- picrylhydrazyl) DPPH in methanol. Incubate the solution in the dark chamber at 37^0c for 15 mins and was measure the changed absorbance at 517 nm using spectrophotometer. DPPH % of free radical scavenging activity of each concentration was calculated and to determine the IC_{50} values. The % inhibition all extracts was calculated as:

Inhibition % = [absorbance (blank) – absorbance (sample)/absorbance (blank)] x100

Anti-Inflammatory Studies

Protein denaturation method

Protein denaturation technique *in vitro* was approved in one of the study by sakat *et al*. The antiinflammatory activity of extracts (PE, EAE and ME) of root was deliberate by using protein denaturation inhibition method. All three extracts were dissolved in DMSO to obtain different concentrations of (500, 250 and 125) µg/ml. 5ml is the reaction mixture consist of egg albumin 0.2 ml (From fresh egg of hens), phosphate buffered saline is 2.8ml (Ph:6.4) and 2ml of concentration of plant extracts. Double distilled water served as control in Similar volume. The mixtures were incubated at 37±2 °C in in an incubator for 15 minutes and then heated at 70 ºC for 5 minutes. After cooling, their absorbance was measured at by using vehicle as blank. The final concentration of diclofenac at [1mg/ml] was used as standard drug and preserved equally for determination of absorbance [8]. The inhibition % of protein denaturation was calculated using the formula given below

$$\textbf{\% inhibition} = \frac{\text{Absorbance control-Absorbance of sample}}{\text{Absorbance Control}} \text{ X } 100$$

Membrane Stabilization Method

The anti-inflammatory activity of three root extracts (PE, EAE and ME) was evaluated by in- vitro RBC membrane stabilization technique. Three extracts were dissolved in DMSO to obtain concentrations of 500µg/ml, 250 µg/ml and 125 µg/ml. Blood was collected and mixed with equal volume of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 ml) and centrifuged with isosaline. To 1ml of HRBC suspension, three different concentrations was added in equal volume of test drug. All the analyzed mixtures were incubated at (37 ^{0}C)for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was The haemoglobin content in the supernatant solution was predictable by using at 560 nm [9]. The haemolysis % and calculate the protection percentage using the formula as followed

$$\% \text{ of haemolysis} = \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

The percentage of protection can be hence calculated from the equation as given below:

$$\% \text{ of protection} = 100 - \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

Here "test OD" is optical density or the test sample's absorbance and "control OD" is optical density or absorbance of the negative control. Here, the Alsevers solution used as a -ve control with blood in it and its not cantained diclofenac or plant Solvent extract in it [9].

Result and Discussion

Anti-microbial studies

Antibacterial studies

Out of all 3 extracts tested EAE exposed well inhibitory volume at 250 µg/ml compared to all the organisms. Where as in case of *S. typhi*, EAE has inhibited very efficiently

showing zone more than standard. Followed by EAE and PE has revealed worthy inhibition in E.coli, *S.typhi*, *P.syringae* and *S.aureus.* where as ME has shown less inhibition zones with all organisms taken for study when compared to EAE and PE as shown in table 1 and below figures 1,2.

Table 1: The different extracts of anti-bacterial activity against various bacterial pathogens.

Extracts	*E. coli*			*S. typhi*			*P. syringae*			*S. aureus*		
µg/ml	500	250	125	500	250	125	500	250	125	500	250	125
PE	13	12.5	13	8	7.5	8	10	9.5	9	11	11	11
EAE	14.5	14	12.5	9	8.5	8	11	10.5	10.5	12	11.5	11
ME	12	11.5	11	7	6.5	7	9	8	8	10	9.5	10
Ciprofloxacin 10 µg/ml	15			8			12			12		

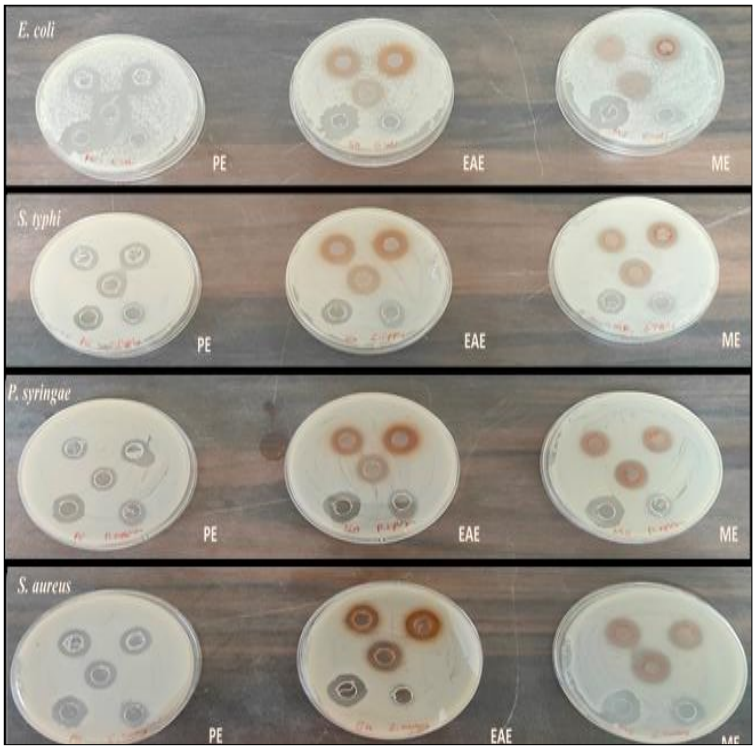

Fig 1: Antibacterial Activity of *Parkia biglandulosa* root extract showing Zone of Inhibition (ZoI) against pathogenic bacterial strains (*E. coli, S. Typhi, P. syringae, S. aureus*) and 3 extracts (Petroleum ether, Ethyl acetate, Methanol). 5 wells representing 3 concentrations- (500, 250 and 125) µg/ml, Ciprofloxacin and DMSO

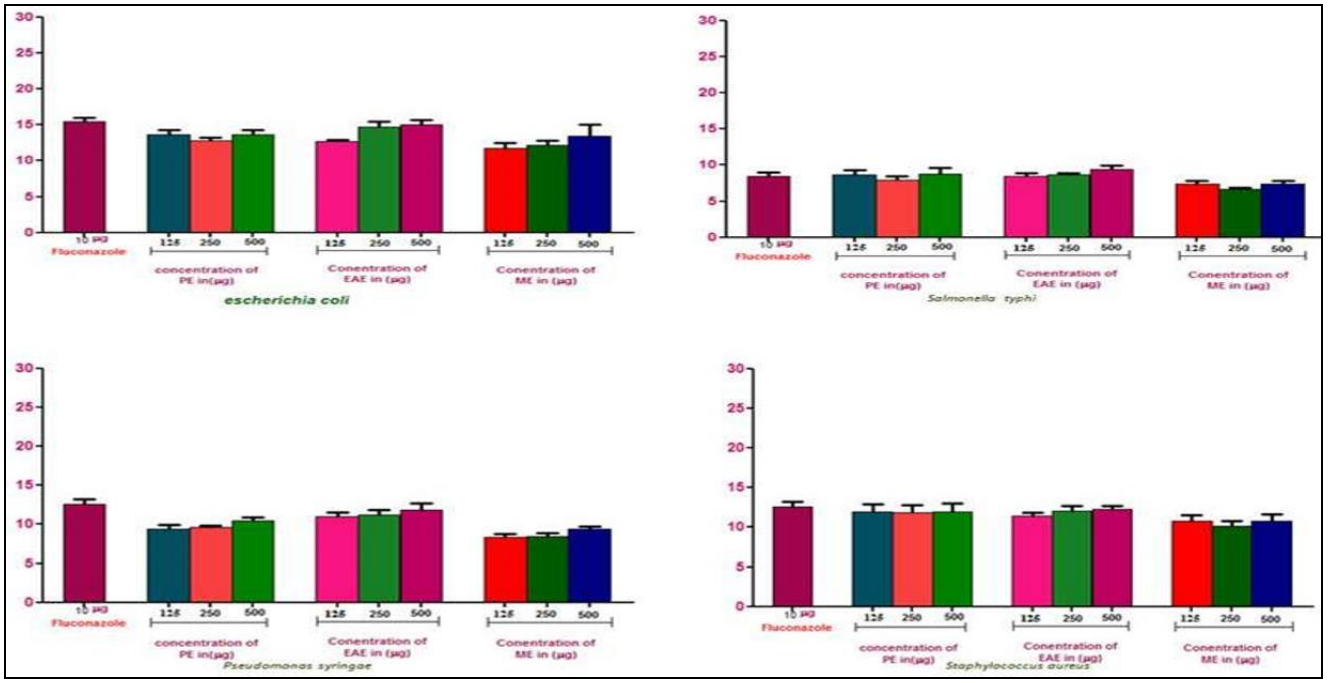

Fig 2: Anti-bacterial activity of three root extracts of *Parkia biglandulosa*

Antifungal studies

Out of all 3 extracts tested EAE exhibited good inhibitory capability at 500 **µ**g/ml counter to 4 pathogenic fungal strains. Followed by EAE, ME has showed good inhibition in *all strains.* Whereas other compounds has not inhibited the growth of both the organisms taken for study. As shown in the table 2 and figure 3, 4.

Table 2: Antifungal activity of the different extracts against various bacterial pathogens.

Extracts	*Aspergillus terrus*		*Penicillium brocae*		*Aspergillus flavus*				*Curvularia*			
µg/ml	500	250	125	500	250	125	500	250	125	500	250	125
PE	13	12.5	13	8	7.5	8	10	9.5	9	11	11	11
EAE	14.5	14	12.5	9	8.5	8	11	10.5	10.5	12	11.5	11
ME	12	11.5	11	7	6.5	7	9	8	8	10	9.5	10
Fluconazole 10 µg/ml	15		8		12				12			

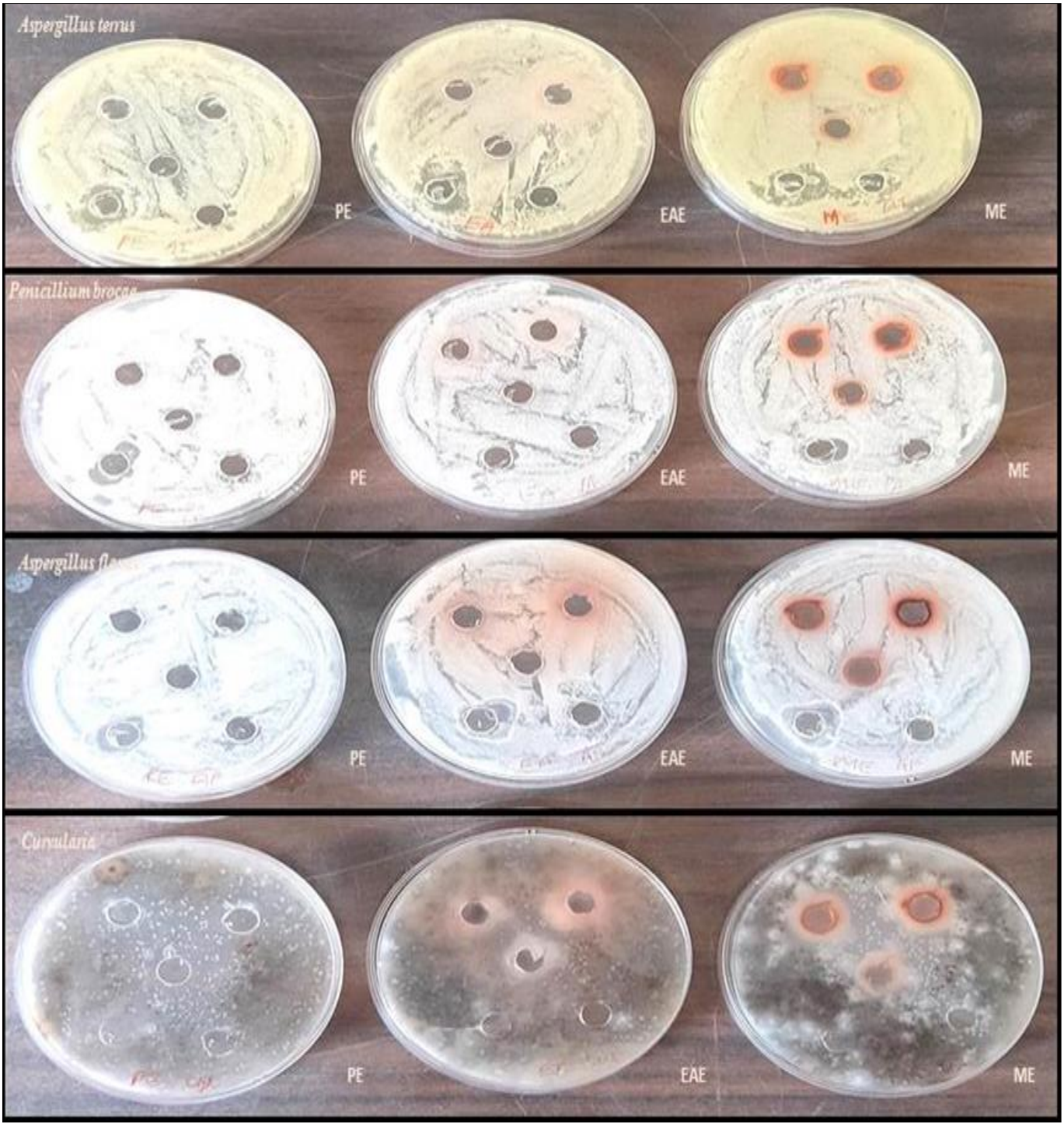

Fig 3: Antifungal Activity of *Parkia biglandulosa* root extract showing Zone of Inhibition (ZoI) against pathogenic organisms. (*Aspergillus terrus*, *Penicillium brocae*, *Aspergillus flavus* and *Curvularia*) and 3 extracts (Petroleum ether, Ethyl acetate, Methanol). 5 wells representing 3 concentrations- (500, 250 and 125) µg/ml, Fluconazole and DMSO.

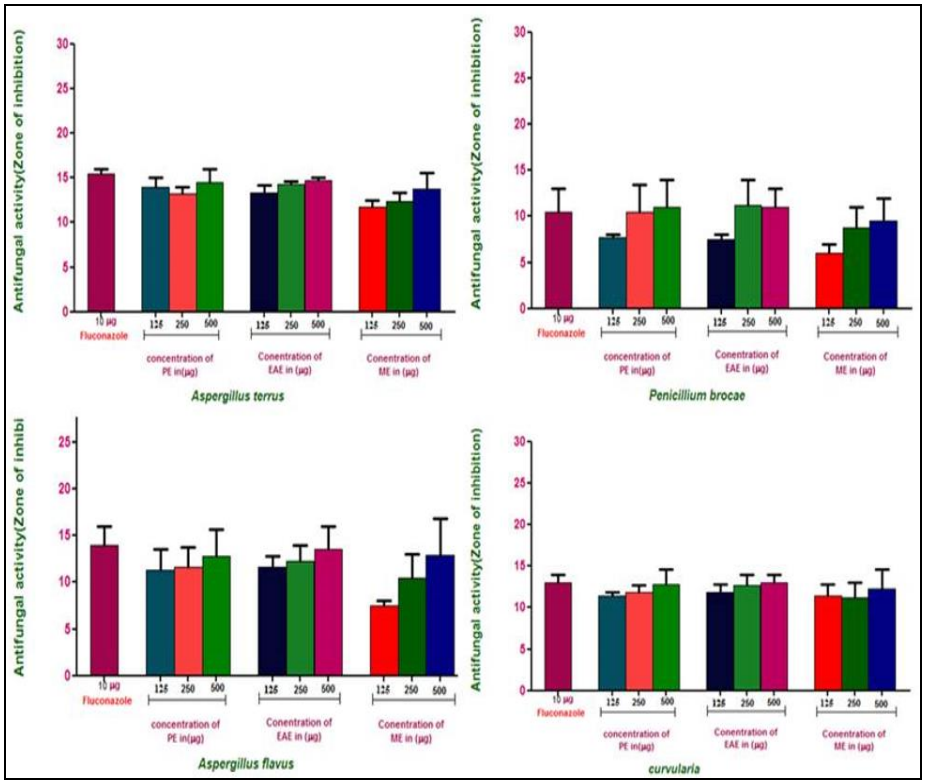

Fig 4: Anti-fungal activity of three root extracts of *Parkia biglandulosa.*

Antioxidant Activity

DPPH assay

Free radical scavenging activity by DPPH of root extracts was projected by using Gallic acid solution as standard. The absorbance data (517nm) were recorded against the selected concentrations (1, 2, 3, 4, 5 μg/ml).

Inhibition concentration and IC50 values for standard (Gallic acid) and the root extracts were recorded in the table 3 respectively represents the anti-oxidant potential of the standard and extracts.

out of five (RPE, RCE, REAE, RME and RDWE). Ethyl acetate extract (2.905) and methanol extract (2.905) show rich IC 50 values compared to standard IC50 values. Which was showed in the table 3 and figure 5

Table 3: DPPH Assay %inhibition and IC_{50} values of root extracts of *Parkia biglandulosa*

Root Extracts		%inhibition(μg/ml)					
SL. No	conc.in μg/ml	GA	RPE	RCE	REAE	RME	RDWE
01	1	33.3	2.7	5.6	13.7	32.9	2.5
02	2	60.2	3.3	9.9	34.4	59.8	5.1
03	3	67.6	5.2	14.4	55.4	64.8	7.0
04	4	75.4	5.9	17.4	71.9	74.6	9.8
05	5	82.2	8.8	19.6	82.9	86.8	11.4
	IC50	1.80	33.28	13.315	2.905	1.876	22.04

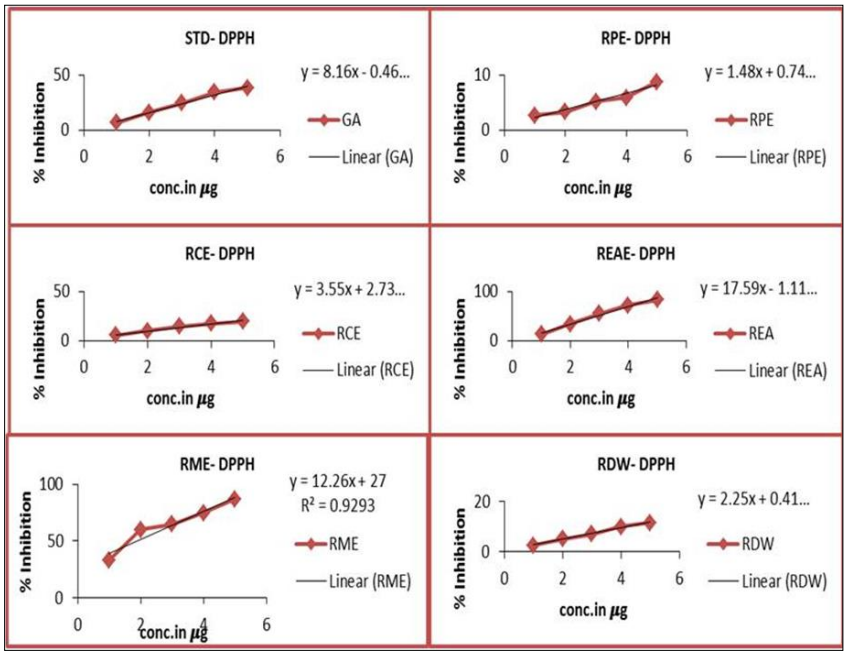

Fig 5: % inhibition DPPH Assay of Root extracts.

ABTS Assay

Free radical scavenging analysis by ABTS of root extracts was assessed by consuming Gallic acid solution as standard. The absorbance data (734nm) were recorded against the selected concentrations (1, 2, 3, 4, 5 μg/ml). Inhibition concentration and IC50 values for standard (Gallic acid) and the root extracts were recorded in the table 4 respectively represents the anti-oxidant potential of the standard and extracts.

Out of five (RPE, RCE, REAE, RME and RDWE). Chloroform extract (2.50) and methanol extract (2.040) show rich IC 50 values compared to standard IC50 values. Which was showed in the table 4 and figure 6

Table 4: ABTS Assay %inhibition and IC_{50} values of root extracts of *Parkia biglandulosa*

Root Extracts		%inhibition(μg/ml)					
SL.NO	conc.in μg/ml	GA	RPE	RCE	REAE	RME	RDWE
01	1	36.8	12.2	38.2	21.9	37.2	12.2
02	2	51.4	13.6	48.0	32.9	54.4	13.6
03	3	60.8	14.8	50.0	45.7	57.8	17.4
04	4	74.6	16.4	64.4	66.4	68.5	20.8
05	5	89	17.3	68.7	67.6	78.0	23.6
	IC50	2.02	30.03	2.50	3.24	2.040	13.82

GA-Gallic Acid, RPE- Root Petroleum ether Extract, RCE-Root Chloroform Extract, REAE-Root Ethyl Acetate Extract, RME-Root Methanol Extract, RDWE- Root Distilled Water Extract.

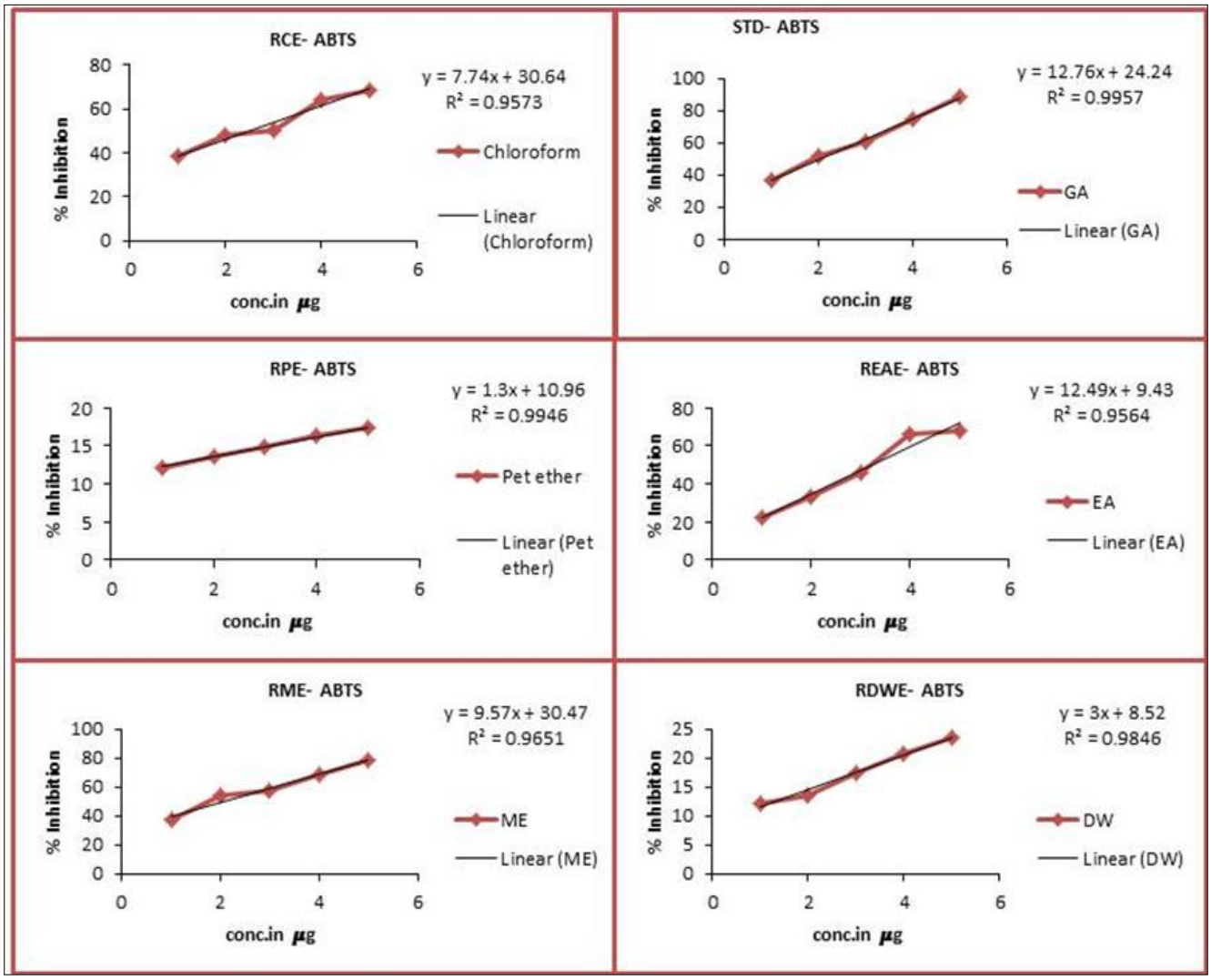

Fig 6: % inhibition ABTS Assay of Root extracts.

Anti-inflammatory studies

Among three extracts taken for study PE and ME has exhibited excellent activity which is very much comparable with standard. ME has shown significant activity followed by PE when compared with standard drug Diclofenac. The extracts have shown effect in dose dependent manner. Activity has increased from 125µg/ml to 250µg/ml to 500µg/ml in inhibition of protein denaturation, percentage of haemolysis, and percentage of protection from as presented in the table 5 and figure 7, 8 and 9.

Table 5: The root extracts shows the anti-inflammatory activity of *Parkia biglandulosa*.

Root Sample	Inhibition of protein denaturation (%)			Percentage of Haemolysis (%)			Percentage of Protection from Haemolysis (%)		
µg/ml	500	250	125	500	250	125	500	250	125
PE	26.6	25.5	23.73	65.54	64.85	61.29	38.4	36.45	35.39
EAE	17.52	15.23	13.15	85.5	81.35	81.05	7.3	6.5	6.6
ME	30.26	28.85	27.21	62.12	60.15	59.12	41.9	40.48	39.35
Diclofenac	30.02			58.12			43.1		

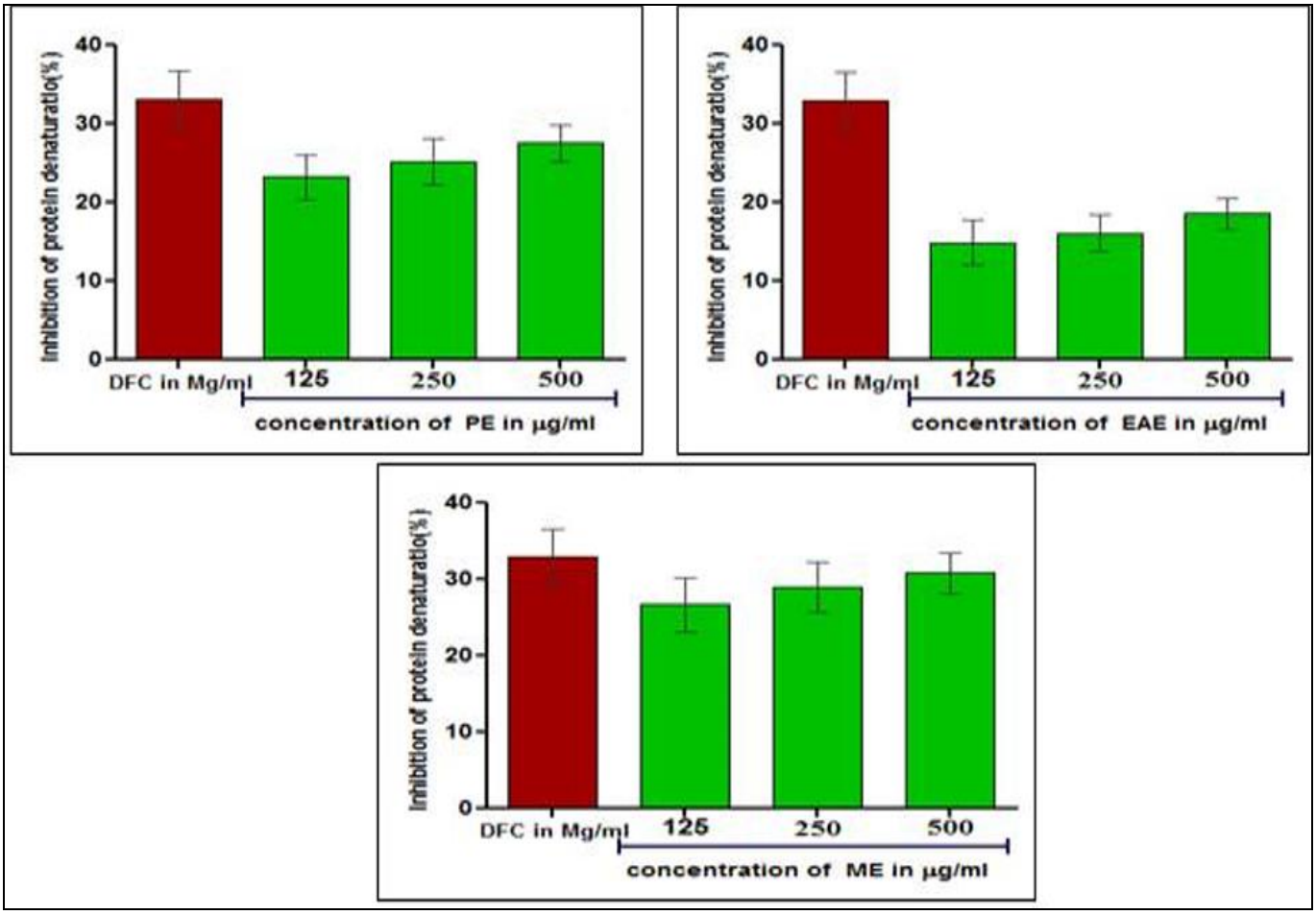

Fig 7: % inhibition of protein denaturation of root extracts

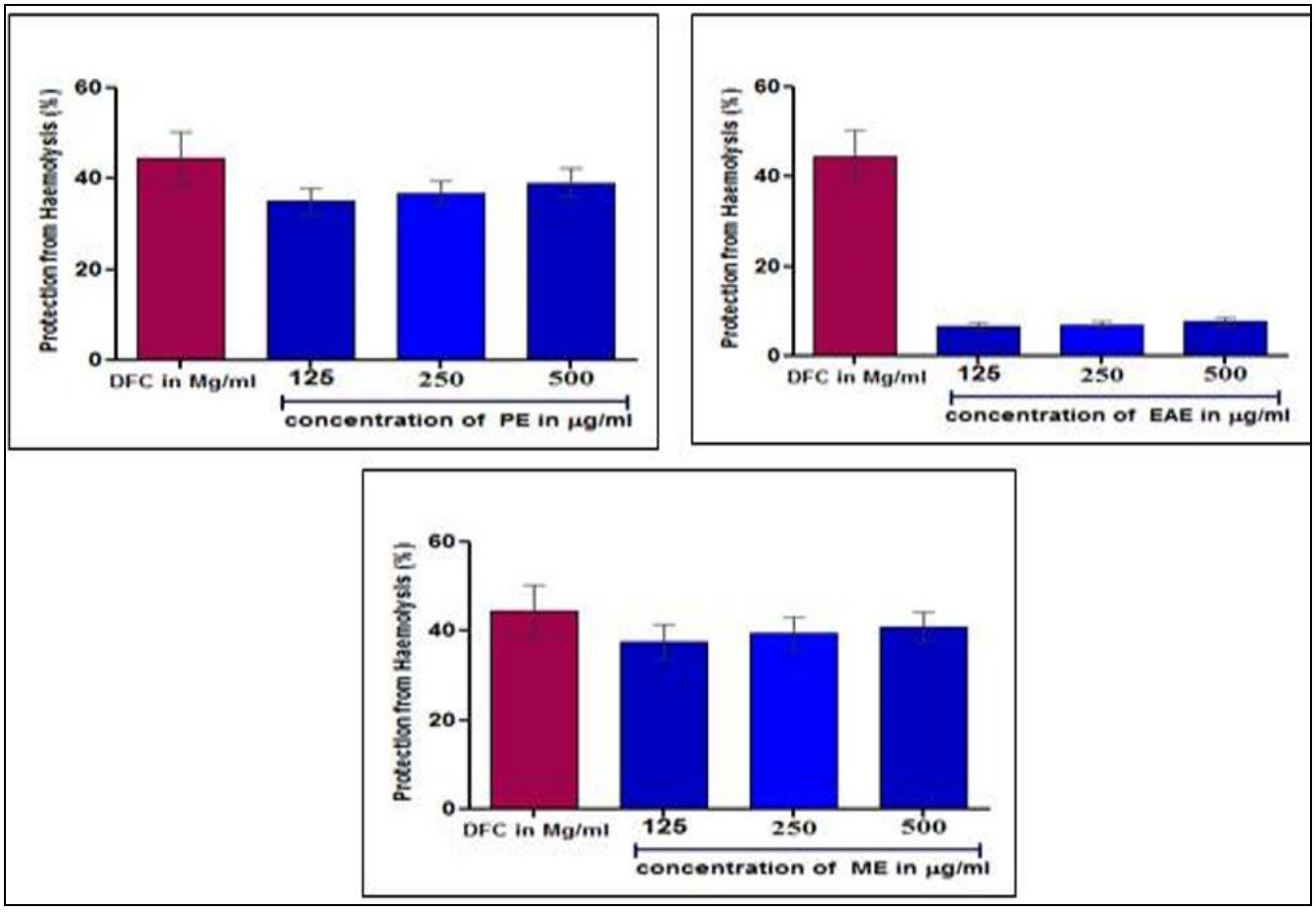

Fig 8: % Protection from haemolysis of root extracts

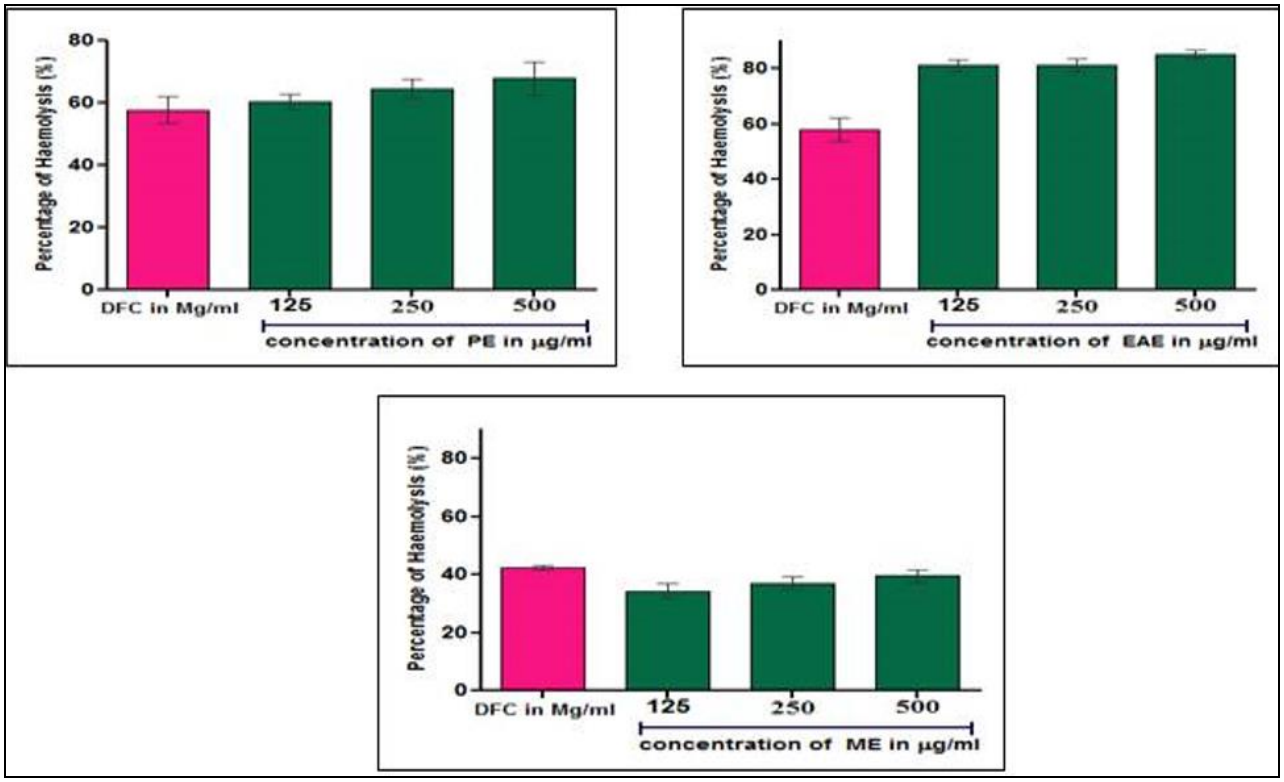

Fig 9: % of Haemolysis of root extracts.

Conclusion

Parkia biglandulosa is one such a medicinal plant wide spread in India. From this study, plant has prospective anti-microbial, anti-oxidant and anti-inflammatory activity. This plant is important source of various phytochemicals with pharmaceutical potential. The root extracts revealed persuasive action against numerous activities shows that the plant become a good source to prepare new drug to treat several diseases and disorders.

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