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Revealing the Phytochemical Profile and Antioxidant Potential of a Medicinal Folklore: Bombax Ceiba L.

REVEALING THE PHYTOCHEMICAL PROFILE AND ANTIOXIDANT POTENTIAL OF A MEDICINAL FOLKLORE: *BOMBAX CEIBA* L.

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ABSTRACT

Current study intends to investigate the extraction yield, phytochemical analysis and antioxidant potential of selected plant by executing a wide range of *in vitro* assays. This was done to suggest the most proficient solvent system and plant part for isolation and purification of probable bioactive leads. The colorimetric estimation of total phenolic, flavonoid contents, total antioxidant capacity, total reducing power and free radical scavenging activity was done. The specific polyphenols were quantified by RP-HPLC analysis. The present findings proved acetone and methanol (AC:M) extract of root bark part as an enriched source of antioxidant phytochemicals. The highest amount of rutin was detected in methanol (M) extract of root bark i.e. 0.70 µg/mg DW followed by methanol (M) extract (0.52 µg/mg DW) of leaf. *Bombax ceiba* L. was found to be a reservoir of phytochemicals possessing antioxidant potential.

Key Words: *Bombax ceiba* L., Phytochemical, Antioxidant.

INTRODUCTION

The world is honored with affluent abundance of therapeutic herbs which have incredible role in alleviating pain and suffering of living beings and enhancing the quality of life (Bora and Sharma, 2011). Undoubtedly indigenous utilization of plants is limitless, yet it is important to find the pharmaceutically vital constituents for protection against fatal diseases. Presently, 25% of pharmaceuticals being utilized by developed nations have been acquired either from plants or their derivatives (Hamayaun and Khan, 2003). Hence, traditional plants are considered as effective remedies against management of different ailments (Rodriguez-Frogoso and Reyes-Esparza, 2008). The use of medicinal plants to fight against resistant infectious diseases led interest in research for discovery of new bioactive leads. Over 100 new drug products as anticancer and anti-infective agents are in clinical development (Harvey, 2003). Therefore, there is a renewed interest in using plants as a source of therapeutic agent and to isolate bioactive compounds for semisynthesis of novel natural products (Kohen and carter, 2005). "*Bombax*" (Greek word; silkworm) "*ceiba*" (silk cotton tree) is a medicinal plant locally called "Sumbal" in Pakistan (Family *Bombacaceae*). It is a fast

growing, strong tree which attains about 40 m of height(Gupta, 2012; Sint et al., 2013). In December, it sheds all leaves and in January it blossoms into bright crimson flowers. It grows best in valleys and in regions where rainfall is about 60 to 450 cm annually (Rameshwar, 2014). The *B. ceiba* L. is a tropical and subtropical tree native to South and East Asia, Indian subcontinent, western Africa and Northern Australia. It is naturally distributed in Myanmar, Pakistan, India, Taiwan, China, Moluccas, Philippine, Java, Borneo, Sulawesi, Lesser Sunda Island, and New Guinea (Sint et al., 2013). Undertaken study aims to evaluate the phytochemical profile and antioxidant potential of *Bombax ceiba* L.

Extraction

Plant was collected and sorted to remove unwanted substances, rinsed with tap water and shade dried at room temperature for 3-4 weeks. The dried parts were pulverized separately by commercial miller to coarse powder. The sonication aided maceration technique was employed for extraction by using fourteen different solvents either alone or 1:1 combination. The accurately weighed plant powder (50 g) was soaked in 200 ml solvent using Erlenmeyer flask at room temperature for 72 hrs with frequent agitation on ultrasonic bath (temperature 25°C, frequency 25 kHz). After 3 days, plant material was strained by muslin cloth and then filtered through Whatmann No. 1 filter paper. Finally, filtrates were concentrated (at room temperature) and dried in vacuum oven (Mermant, Germany). The crude extracts were then stored at -20°C. The different solvents employed for extraction process included; n-hexane (NH), chloroform (CH), ethyl acetate (EA), chloroform : methanol (CH:M), chloroform : ethanol (CH:E), acetone : ethyl acetate (AC:EA), methanol : ethyl acetate (M:EA), ethanol : ethyl acetate (E:EA), acetone (AC), methanol (M), ethanol (E), acetone : distilled water (AC:W), methanol : distilled water (M:W) and distilled water (W).

Extract recovery

The percent extract recovery was calculated by using the following equation:

$$\% \text{ extract recovery} = (W_e / W_p) \times 100$$

Where:

W_e = crude extract weight (of each solvent system)

W_p = dried powder weight i.e. (50 g)

MATERIALS AND METHODS

Phytochemical analysis

RP-HPLC quantitative analysis

The polyphenols were detected and quantified in *B. ceiba* crude extracts using standard protocol for reverse phase high performance liquid chromatography (RP-HPLC) analysis (Fatima et al., 2015).

Total phenolic content (TPC)

TPC was estimated using Folin-Ciocalteu reagent method with slight modifications as discussed previously (Ahmed et al., 2017). Analysis was performed in triplicate expressing results as µg gallic acid equivalent per mg dry weight (µg GAE/mg DW).

Total flavonoid content (TFC)

The total flavonoid content was assessed by adopting the previously reported aluminium chloride method with slight changes (Ahmed et al., 2017). The assay was run

thrice and results were expressed as µg quercetin equivalent per mg of plant dry weight (µg QE/mg DW).

Antioxidant assays

Total antioxidant capacity (TAC)

The phosphomolybdenum based assay was used to determine antioxidant capacity of test extracts (Zahra et al., 2017). The results were expressed as µg equivalents of ascorbic acid per mg of dry weight (µg AAE/mg DW).

Total reducing power (TRP)

The reducing power of different test extracts was estimated using a previously revealed potassium ferricyanide colorimetric assay (Zahra et al., 2017). The results were expressed as µg ascorbic acid equivalent per mg dry weight (µg AAE/mg DW). The assay was repeated thrice.

Free radical scavenging assay (FRSA)

The radical scavenging potential of test extracts was determined by using 2, 2-diphenyl 1-picrylhydrazyl (DPPH) method (Haq et al., 2010). The experiment was repeated thrice by using ascorbic acid as a positive control. The IC_{50} for the crude extracts showing ≥ 50% FRSA was calculated using three fold serial dilutions (from 7.40-200 µg/ml).

RESULTS AND DISCUSSION

A total of 84 extracts were prepared by applying fourteen different solvent combinations on six parts of *B. ceiba* L. The percent extract recovery (% w/w) is given in Table 1.

Table 1: Extract recovery of different parts of *B. ceiba* L.

Extract codes	App. Polarity index	Percent extract recovery (% w/w)					
		Flower	Leaf	Stem bark	Stem wood	Root bark	Root wood
NH	0.01	0.24	0.74	0.44	0.14	0.28	0.14
CH	4.10	0.74	2.14	0.44	0.34	0.64	0.84
EA	4.40	0.74	1.14	0.44	0.24	1.04	0.64
CH:M	4.60	3.04	2.94	2.44	1.34	4.44	1.34
CH:E	4.65	2.14	2.54	1.74	1.04	1.84	1.14
AC:EA	4.75	2.84	1.34	3.84	0.34	0.34	0.94
M:EA	4.75	3.24	3.24	4.24	0.94	8.14	1.84
E:EA	4.80	2.04	4.74	3.04	0.94	4.94	1.34
AC	5.10	3.04	3.94	5.04	1.34	4.74	1.94
M	5.10	5.14	4.24	5.74	2.04	7.44	2.14
E	5.20	2.94	4.14	4.24	1.14	5.74	1.34
AC:W	7.05	2.94	2.6	10.14	3.84	14.24	3.74
M:W	7.05	2.64	11.94	9.24	3.94	12.24	3.94
W	9.00	5.04	6.4	7.94	1.44	2.54	1.14

Percent extract recovery of *B. ceiba* L. using mono and binary (1:1) solvents for extraction. NH= n-hexane, CH= chloroform, EA= ethyl acetate, CH:M= chloroform : methanol, CH:E= chloroform : ethanol, AC:EA= acetone : ethyl acetate, M:EA= methanol : ethyl acetate, E:EA= ethanol : ethyl acetate, AC= acetone, M= methanol, E= ethanol, AC:W= acetone : distilled water, M:W= methanol : distilled water and W= distilled water.

The extraction yield varied proportionally with the polarity of solvent system which shows the impact of solvent polarity index on the extractable components of the dried plant material (Fatima et al., 2015). The maximum yield of 14.24% w/w in AC:W extract of root bark part was recorded while n-hexane extract of stem wood and root wood parts exhibited the least yield (0.14% w/w). For convenience, the extracts were subdivided into three categories (on the basis of polarity indices); nonpolar (polarity index from 0-4.5), moderately polar (polarity index from 4.6-7.0) and polar (polarity index from 7.1-9.0) extracts.

It was observed from the HPLC quantitative analysis (as shown in Figure 1) that the highest amount of rutin was detected in M extract of root bark i.e. 0.70 µg/mg DW followed by M extract (0.52 µg/mg DW) of leaf (Table 2).

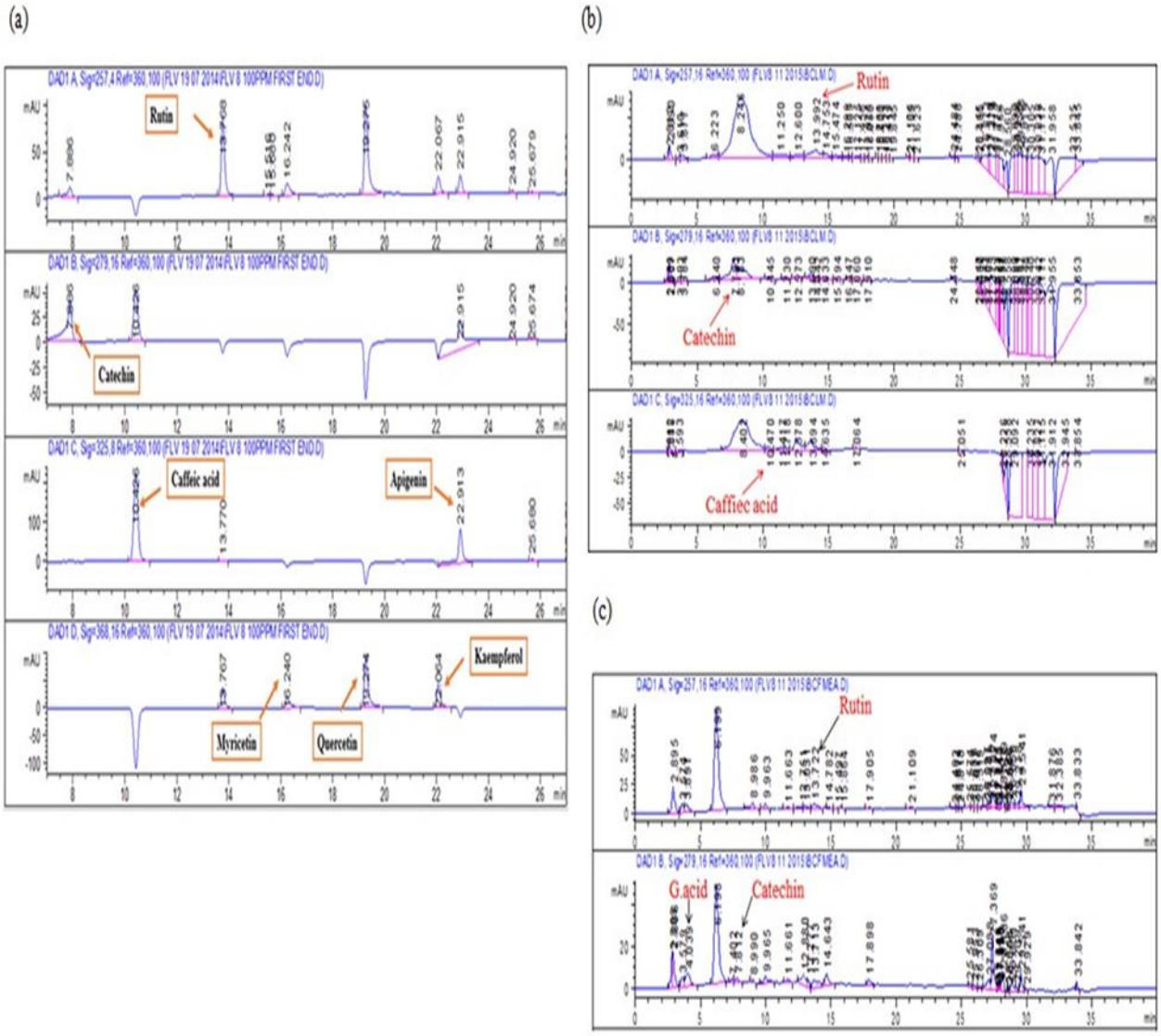

Figure 1: RP-HPLC chromatograms of (a) standard polyphenols (b) M extract of leaf (c) EA:M extract of flower of *B. ceiba* L. Note: G. acid = gallic acid

Table 2: Polyphenols quantified in six different parts of *B.ceiba* L. by RP-HPLC analysis.

Extract codes	Polyphenols (µg/mg DW)							
	Rutin	Gallic acid	Catechin	Caffeic acid	Apigenin	Myrecetin	Quercetin	kaempferol
Flower								
M	0.01	0.04	0.03	---	---	---	---	---
E	0.03	0.03	0.02	---	---	---	---	---
EA	0.002	---	0.003	---	---	---	---	---
M:EA	0.05	0.03	0.02	---	---	---	---	---
Leaf								
M	0.52	---	0.37	0.007	---	---	---	---
E	0.23	---	0.49	0.005	---	---	---	---
EA	0.006	---	---	---	---	---	---	---
M:EA	0.50	---	0.41	---	---	---	---	---
Stem bark								
M	0.22	---	0.48	0.002	---	---	---	---
E	0.37	---	0.00	0.001	---	---	---	---
EA	---	---	---	---	---	---	---	---
M:EA	0.20	---	0.42	0.001	---	---	---	---
Stem wood								
M	0.02	---	0.11	---	---	---	---	---
E	0.01	---	0.04	---	---	---	---	---
EA	0.005	---	---	---	---	---	---	---
M:EA	0.03	---	0.03	---	---	---	---	---
Root bark								
M	0.70	---	---	0.007	---	---	---	---
E	0.35	---	---	0.006	---	---	---	---
EA	---	---	---	---	---	---	---	---
M:EA	0.36	---	0.38	0.009	---	---	---	---
Root wood								
M	0.02	---	---	---	---	---	---	---
E	0.002	---	---	---	---	---	---	---
EA	---	---	---	---	---	---	---	---
M:EA	---	---	---	---	---	---	---	---

Note: --: not detected, M = methanol, E = ethanol, EA = ethylacetate, and M:EA = methanol: ethylacetate

Rutin was quantified in all the parts tested. The highest amount of catechin was quantified in leaf E extract i.e. 0.49 µg/mg DW followed by stem bark M extract with 0.48 µg/mg DW. Gallic acid and catechin were also detected in minute amounts whereas apigenin, quercetin, kaempferol and myricetin were not detected at all. Rutin is a well-studied metabolite of plants with excellent hepatoprotective, antiinflammatory

and chemopreventive activities (Kubola et al., 2011).

Another well-known polyphenol compound catechin has an immense antioxidant potential and offers a protective barrier against free radicals. Reports are evident that catechin has defensive role against neurological issues, inflammation and apoptosis (Sutherland et al., 2006) So the present study clearly validates the antioxidant potential of the plant which is attributable to the presence of rutin, catechin and numerous other constituents unknown at this point. The polyphenols including phenolic acids, tannins and flavonoids have a promising role in oxidative stress (Karimi et al., 2015). Phenolic compounds are extremely diverse secondary metabolites with a variety of pharmacological properties. The studies have shown that phenolic compounds show antioxidant behavior by single oxygen quenching, hydrogen donation, metal ion chelation, free radical scavenging, or act as substrate for attack by superoxide. Phenolics also terminate the free radical reactions and therefore halt lipid peroxidation (Robards et al., 1999).

According to plant part, results of TPC assay (Figure 2) decrease in the following order; root bark > stem bark > leaf > flower > root wood > stem wood with highest value of root bark's AC:W extract (30.61 ± 0.7 µg GAE/mg DW). While TFC values (Figure 2) sequentially decline as; leaf > root bark > stem bark > flower > root wood > stem wood with highest value of leaf's E:EA extract (1.82 ± 0.015 µg QE/mg DW) followed by root bark's AC:W extract (1.23 ± 0.02 µg QE/mg DW).

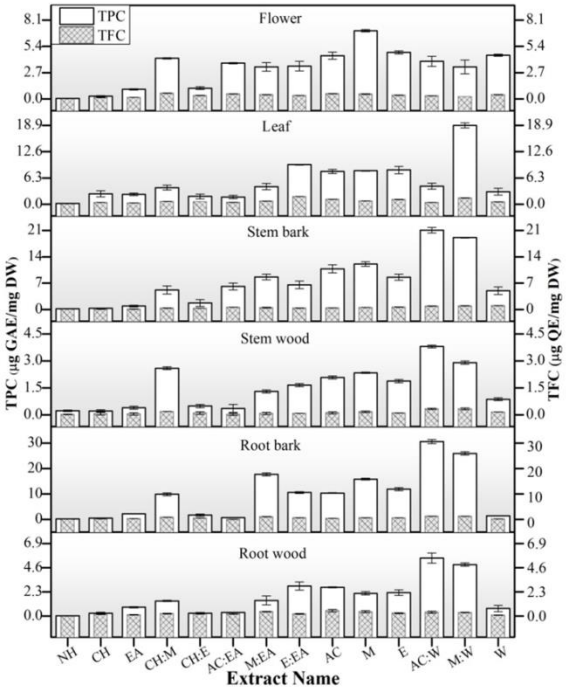

Figure 2: Comparison of total phenolic content (µg GAE/mg DW) and total flavonoid content (µg QE/mg DW) among six parts of *B. ceiba* L. Values are presented as mean ± Standard deviation (*n* = 3).

The descending trend in the TAC results of *B. ceiba* parts as; root bark > stem bark > leaf > stem wood > flower > root wood and that of TRP results as; root bark > stem bark > root wood > stem wood > leaf flower. The most prominent TAC and TRP activities (as given in Figure 3) were recorded in root bark's AC:W extract (28.01 ± 1.11 and 31.60 ± 3.75 µg AAE/mg DW respectively). In the same way, percent free radical scavenging activity (% FRSA) of each plant part (as shown in Figure 4) exhibited the following trend; root bark > stem bark > flower > leaf > root wood > stem wood. Most of the moderately polar extracts of root bark part (such as M:EA, E:EA, AC, M, and E) exhibited notable % FRSA with IC_{50} estimation of 9.5 to 11.5 µg/ml. However, root bark's AC:W extract displayed comparable IC_{50} 21.21 ± 0.73 µg/ml as of standard ascorbic acid (21.8 ± 0.23 µg/ml).

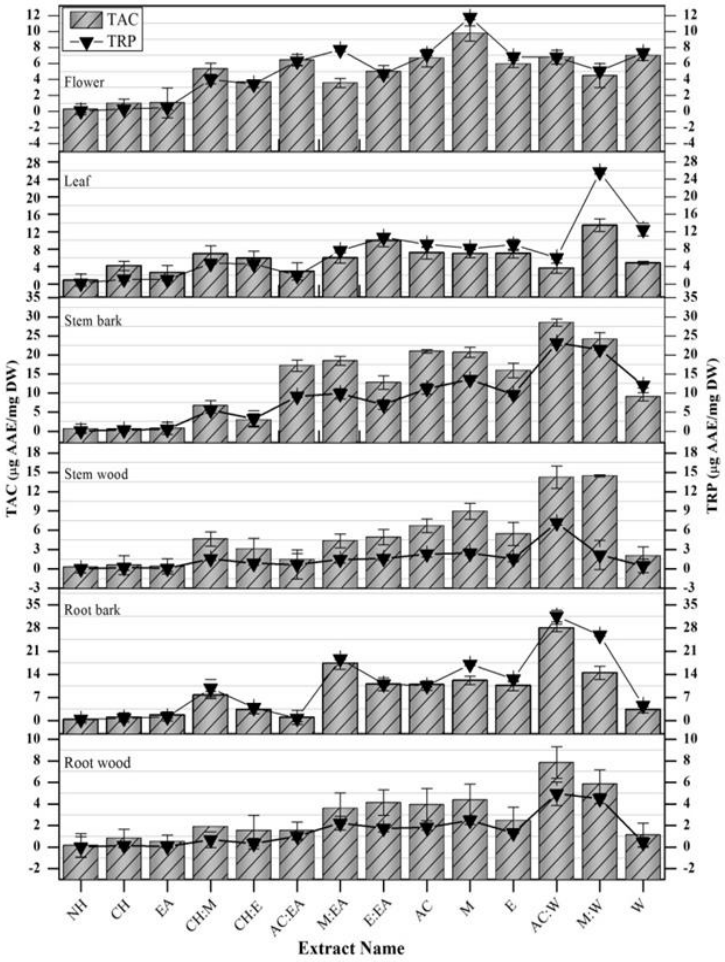

Figure 3: Comparison of total antioxidant capacity (µg AAE/mg DW) and total reducing power (µg AAE/mg DW) among six parts of *B. ceiba* L. Values are presented as mean ± Standard deviation (*n* = 3).

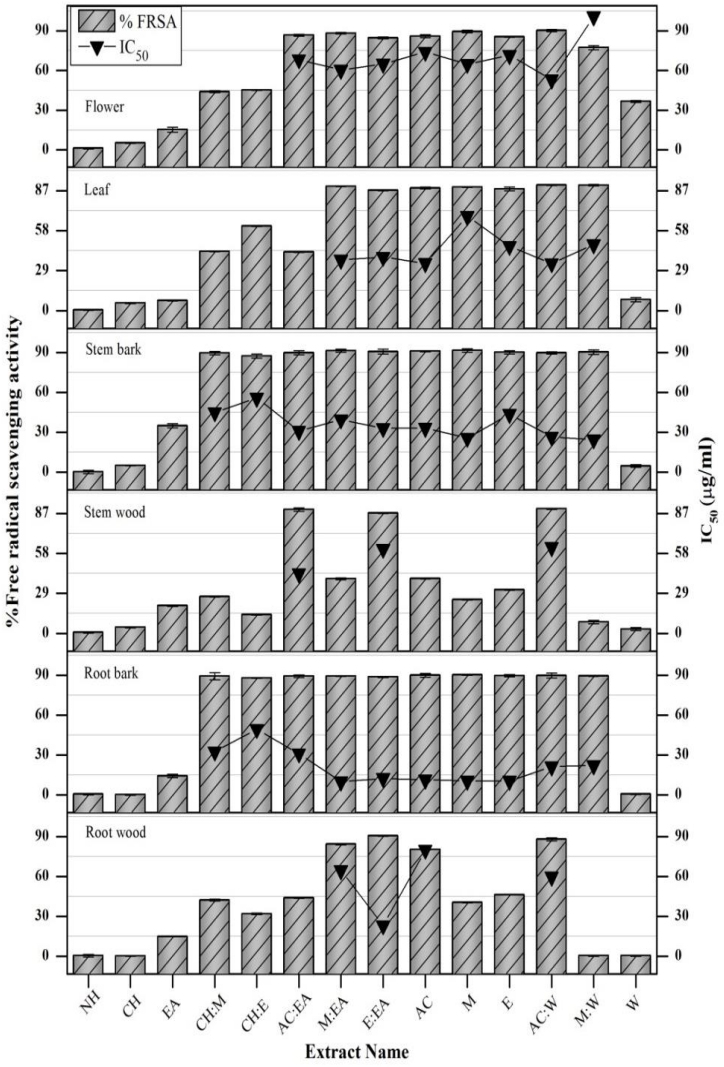

Figure 4: Presentation of % FRSA and IC_{50} among six parts of *B. ceiba* L. Missing values indicate IC_{50} > 200 µg/ml. IC_{50} of ascorbic acid is found to be 21.8 ± 0.23 µg/ml. Values are presented as mean ± Standard deviation (*n* = 3).

Highly considerable correlation (Table 3) can be seen between TPC and TAC (R^2 value 0.9055) and TPC and TRP (R^2 value 0.9882) which proves that the phenolic compounds are responsible to the great extent for antioxidant behavior. While TFC also gave a moderate corelation (R^2 value 0.8548) which suggests presence of phenolic compounds other than flavonoid in nature. However, % FRSA gave a minor corelation (R^2 value 0.3615) which denotes radical scavenging activity to compounds other than phenolic in nature. According to literature review, the aqueous and ethanol extracts of

B. ceiba bark were shown to be highly active in total reducing, total antioxidant and % FRSA bioassays (IC_{50} values around 100 µg/ml) (Gandhare et al., 2010) which further proves the antioxidant potential of moderately polar extracts of the subject plant.

Table 3 Correlations of TPC with TFC, and potential antioxidant assays among different parts *B. ceiba.*

Plant Parts	TFC	DPPH	TAC	TRP
Leaf	0.6931	0.4207	0.8404	0.8144
Flower	0.6084	0.5760	0.8986	0.8651
Stem Wood	0.7399	0.1112	0.8413	0.7258
Stem Bark	0.5536	0.3539	0.8860	0.9334
Root Wood	0.3380	0.1961	0.8958	0.8800
Root Bark	0.8548	0.3615	0.9055	0.9882

CONCLUSION

The present findings proved acetone and methanol (AC:M) extract of root bark part as an enriched source of antioxidant phytochemicals. Bioguided isolation should be carried out to isolate the bioactive leads. Plant based green synthesis of nanoparticles is recommended because phytochemicals such as terpenoids, flavonoids, sugars, proteins, and alkaloids are responsible for reduction in metal ions resulting in NP formation (Nazli et al., 2018).

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