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ORIGINAL ARTICLE

In vitro Phytochemical Analysis and Antioxidant Assay of Fruit Extracts of *Sapindus mukorossi* Gaertn. and *Acacia concinna* DC

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ABSTRACT

Background: *Sapindus mukorossi,* commonly known as areetha, and *Acacia concinna,* commonly known as Shikakai reported to have potential naturally occurring medicinal properties. The prime medicinal actions included cleaning, therapeutic mode of action, antifungal properties and cited as important ingredients in ayurvedic medicines. The antioxidant potential of these plants could be the best alternative for the commercially synthesized antioxidants added in medicinal and cosmetic products.

Objectives: To examine the variety of phytochemicals and antioxidant potential of fruit extracts of both plants' species.

Methodology: The dry fruits were powdered and extracts of different polar and non-polar solvents were used to examine the presence of secondary metabolites in plants bearing fruits by using phytochemical tests and DPPH assays for antioxidant potential.

Results: The experimental outcomes revealed a positive response in case of Cardiac glycosides, Flavonoids, Coumarins and Terpenoids and showed negative results for Phlobatannins and Anthraquinones, respectively. Positive results were obtained in case of total antioxidant assay as compared to that of DPPH assay, in comparison to the standard antioxidant agents available commercially that were utilized in the study.

Conclusion: This study concluded *Acacia and Sapindus* fruits to be organic replacement for commercially synthesized antioxidants and a potential source of secondary metabolites.

Keywords	*Address of Correspondence	Article info.
Dried Fruits Powder, Medicinal Plants, Phytochemistry, Plant Extracts, Polar, Non-Polar Solvents, Secondary Metabolites.	*faizaanum55@gmail.com*	*Received: July 26, 2021* *Accepted: May 31, 2022*

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INTRODUCTION

Phytochemicals are organic compounds that may act as defense mechanism of plants against different predators[1]. The beneficial and multipurpose pharmacological properties of medicinal flora are fundamentally reliant on their phytochemical components[2]. Various kinds of plants are used for medicinal purposes owing to their medicinal potential[3]. At the present time, a major drawback is that out of the 250,000-500,000 known plant species on the planet earth, only a few plants have been examined phytochemically for their potential properties[4]. Therefore, there is a dire need to perform research on the identification of bioactive compounds that are beneficial for treating numerous human ailments caused by bacterial and fungal infections[5].

Sapindus mukorossi (Reetha) and *Acacia concinna* (shikakai) are potential medicinal plants mostly used in manufacturing of hair care products and detoxifiers[6]. *Sapindus mukorossi* is mostly found in the tropical and subtropical territories of Asia having tropical weather[7]. It is a tall deciduous tree which attains a height of 20m. *Sapindus mukossori* has pharmaceutical properties e.g. antidandruff, mucolytic, and spermicidal. It is utilized in the treatment of diseases like psoriasis (skin infections), chlorosis (a sort of weakness), dermatitis, normal tingles, and in ailment caused by *Helicobacter pylori*, a gut pathogen[8]. *Acacia concinna,* commonly called Shikakai is a remedial plant, the fruit of which is utilized, traditionally known as "fruit for hair"[9]. The plant owns bioactive natural metabolites having anticoagulant (repressing blood coagulation), and antiplatelet properties[10]. The leaves are used for the treatment of diabetes and furthermore, for skin ailments in India, Myanmar, and Thailand[11].

Scientists performed a phytochemical investigation of *Sapindus mukorossi* extracts in water and ethanol which showed the presence of various secondary metabolites for example, phytosterols, flavonoids, alkaloids, phenolics, saponins, tannins, and glycosides[12]. In this research, we have investigated the presence of complex secondary phytometabolites such as triterpenes, saponins, glycosides, flavonoids, alkaloids, tannins, and anthraquinones. Leaf extracts were prepared by using various polar and non-polar chemical solvents. The main aim of this research work was to examine the utilization of medicinal plants depending upon their naturally occurring phytochemical nature and finding their potential for targeted secondary usage.

MATERIALS AND METHODS

Plants selected for examination were phytochemically analyzed and then subjected for an antioxidant assay. Analytical grade chemicals were used in the study for precise reaction chemistry.

Review of Experimental Approach

The plant extracts were stirred on the magnetic stirrer for 5h at room temperature. The spectrophotometric calculations of samples were done by using UV/Vis spectrophotometer (UV-1650PC, Shimadzu, Japan).

Preparation of Reagents

First, 0.6M H_2SO_4 (95-97%) was prepared by dissolving 1.66ml H_2SO_4 in 100ml of distilled water in a volumetric flask. Potassium dihydrogen phosphate was prepared by dissolving 0.3808g potassium dihydrogen phosphate in 100ml of distilled water. Ammonium molybdate (4mM) was prepared by dissolving 0.0784g of ammonium and 0.3g molybdate in 100ml of distilled water, respectively.

Plant Materials

Fruit samples of *S. mukorossi* Gaertn. and *A. concinna* (Willd.) DC. were sorted, washed and extraction was done by utilizing petroleum ether, dis. Water, methanol and chloroform solvents.

Plant Extract Preparation

To make extracts, samples were sun dried, grounded, and the powder was preserved in amber shaded containers at 4ºC.

Solvent Extraction: Maceration

For this, 15g powdered sample was added into 30ml of (non-polar and polar) solvents for at least 7 days (approx. 160hrs). Samples were filtered, isolated, and preserved in glass vials. The leftover was set for next extraction.

Ash, Moisture Content and Acid

Moisture content, total ash, and acid insolubility were determined by using standard methods[13].

Phytochemical Assay

Phytochemical assay was conducted as per standard procedures to estimate the following metabolites[14]:

Alkaloids

Approx. 0.25g of powdered plant material was mixed in 4ml of 1% HCl followed by stirring and heating. Six drops of Mayors reagent were added to the 1ml of plant filtrate displaying creamy orange color compound as an indicator of an Alkaloids.

Frothing Test (Saponins)

Distilled water (5ml) was poured into 0.5g powder plant sample and shaken vigorously to observe frothing intensity.

Coumarins

Initially, 0.5g of plant sample with 0.1N NaOH was added in a secure test tube with filter paper and kept in a water bath for 10min. Later, the filter paper was observed under bright (UV) light for the presence of yellow inflorescence.

Anthraquinones

Here, 3ml of 1% HCL was added into 0.5g of plant sample and filtered, followed by the addition of 2ml benzene and shaken. Benzene on the top was decanted and few drops of 10% ammonium hydroxide were added. The generation of violet, red, or pink color solution demonstrated the indication of anthraquinones.

Terpenoids (Liebermann-Burchard Test)

Plant extract (2ml) was dissolved in 2ml of chloroform and filtered. For the estimation, a drop of sulphuric acid and methanol was added to the filtrate. Appearance of blue-green color ring in the tube confirmed the presence of terpenoids.

Flavonoids

Plant powder (5g) and petroleum ether were dissolved together and mixed in 20ml of 80% ethanol and filtered. Then, 3ml of filtrate was mixed with 4ml of 1% KOH to see dim yellow shade substance indicating flavonoids.

Phlobatannins

Petroleum ether (5ml) and 1% HCL when mixed into 0.25g plant sample generated red precipitates showing the presence of phlobatannins.

Tannins

Iron chloride (1%) and 10ml of distilled water were added to 0.25g sample and filtered. Tannins were indicated with the production of dark green to dark blue tinge color.

Cardiac glycosides (Keller-Kiliani Test)

For this, 0.5g of sample was mixed with 2ml of glacial acetic acid and few drops of 1% iron chloride along with 1ml of extracted sulphuric acid to indicate the presence of cardiac glycosides by the formation of green-blue color.

Assessment of Antioxidant Activity

Total antioxidant and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging was conducted by using standard methods[15]. The transfer of electron is responsible for the reduction of an antioxidant reagent. The sample capacity to scavenge the radicals was observed by spectrophotometer and quantified in BHT (butylated hydroxytoluene) with α-tocopherol equivalents as standards[16].

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Measure

DPPH assay was used to assess the scavenging of free radicals in solvents. Homogenous solutions were prepared by adding 0.5mg/ml solute in DMSO (dimethyl sulphoxide). DPPH and DMSO were mixed and kept for 30min in dry shade.

DPPH radical scavenging was conducted by using spectrophotometer at 517nm absorbance with three replicates. The antioxidant agent action was derived as % as[17]:

$$\text{SC } \% = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{Absorbance of control}} \times 100$$

The control group included all experimental ingredients except the sample however, testing of DPPH scavenging action of BHT and α-tocopherol was done at diverse concentrations, e.g., 0.5, 1, 2.5 and 5mg/ml to observe correlation between them. Statistical software used for analysis were SPSS and Minitab 2.0, respectively.

Total Antioxidant Assay (TAOA) Determination

All solutions with concentration 0.1ml or 0.5mg/ml were shaken up by adding reagent (1.9ml) carrying (4mM $(NH_4)_6Mo_7O_{24}$, 28mM Na_2SO_4 and 0.6M H_2SO_4) and set for

incubation for 1hr at 95°C temperature. Furthermore, the mixture was set for cooling and estimation of wavelength was done at calibrated spectrophotometer set at a wavelength of 695nm along with blank. Similar estimation was conducted for BHT (0.5mg/ml) antioxidant potential.

Statistical Analysis

Mean ± S.E and standard deviation of all triplicates of data sets were calculated and means were compared by using the Turkeys' multiple range tests at a significance level of α=0.5 as probability values. Data sets were further analyzed using two-way ANOVA, in completely randomized design to estimate significant effects of the factors.

RESULTS AND DISCUSSION

Both test species are known to be utilized for the treatment of different ailments since many decades (Table **1**) with high antioxidant potential. Therefore *in vitro* assays were analyzed for total phenolics and flavonoids.

Qualitative Phytochemical Analysis

Plant material was examined for various compounds[21]. Most of the compounds were identified as secondary metabolites that give particular shade and fragrance to the plant. In this study, it has been noticed that every test shows a particular shading as the end point (Table **2** & Fig. **1**).

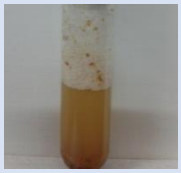
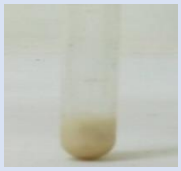
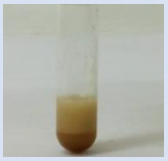
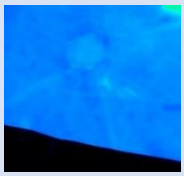
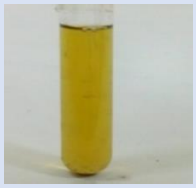
Secondary Metabolites Tests

In the phytochemical investigation of *S. mukorossi* Gaertn and *A. concinna* (Willd.) DC., lack of cream and orange precipitates demonstrated the absence of alkaloids. Formation of froth in the sample analysis indicates the presence of saponins. Absence of white froth indicated the absence of anthraquinones. Emanation of yellowish inflorescence in UV light the demonstrated the presence of coumarins in the examination of *S. mukorossi* and *A. concinna* (Willd.). Fruits formation of blue green ring demonstrated the indication of terpenoids. A light-yellow shading in the extracts demonstrated that flavonoids were present. Sign of earthy green shading demonstrated that tannins are available while the absence of caramel green color indicated that tannins were not present. No deposition of red precipitates demonstrated that phlobotannins are missing in individual plant sample. Sign of blue green color demonstrated that glycosides were present (Table **2**).

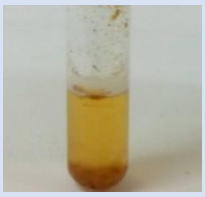
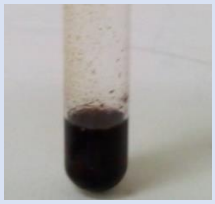
Table 1. Medicinal Plant Species Analysed for the Study.

Scientific Name	Common Name	Family	Growth form	Moisture Content (g/100g)	Ash Content (g/100g)	Acid Insoluble ash/ Silicates (g/100g)	Part analyzed	Fruit	Medical Importance	Reference
Sapindus mukorossi Garten.	Areetha Soap Berry	Spaindaceae	Deciduous Tree	4.24± 0.098	16.96± 0.24	10.7± 0.05	Fruit	Berry	Epidermal Cleanser, Insecticide, headache, migraine, eczema	18, 19
Accacia concinna DC.	Shikakai	Fabaceae	legume	5.07± 0.032	23.7± 0.15	21.03± 0.23	Fruit pod	Pod	Shampoo, natural oil, cleaning agent, detergent, jaundice	20, 11

Table 2. Detection of Secondary Metabolites in Plant Samples.

Phyto constituent	Indication	Result	Images	
			S. mukorossi	*A. concinna*
Alkaloids	Orange color appear	✓		
Saponins	Froth formation occur	✓		
Anthraquinone	White colour frouth	×		
Coumarins	Yellow inflorescence	✓		
Terpenoids	Occurrence of Ring	✓		
Flavaniods	Appearance of yellow color	✓		
Tannins	Brownish green coloration not appeared	×		

Contd….

Phlobotanins	No Red ppt. form	×		
Cardiac glycosides	Violet ring and brown color appear	✓		

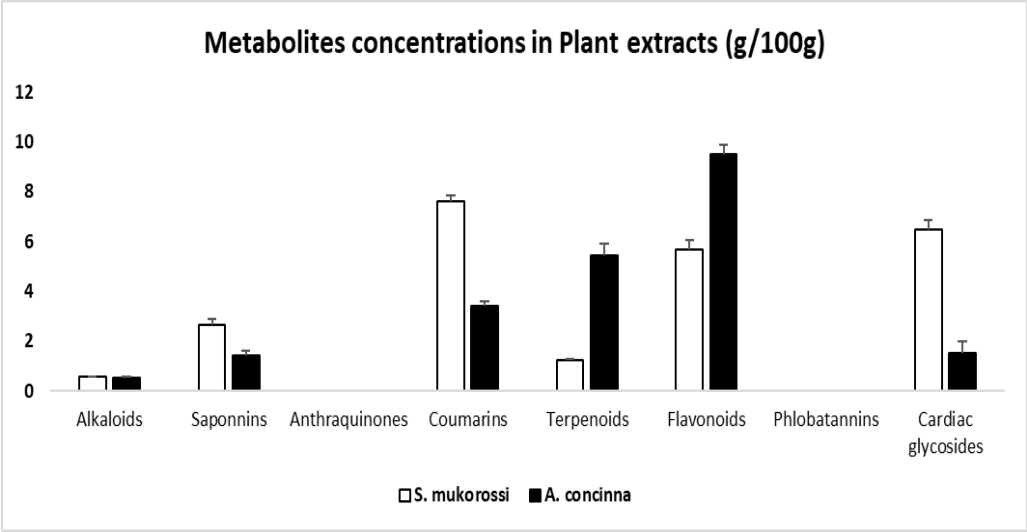


Figure 1. Secondary metabolites concentration g/100g.

Table **2** shows the presence and absence of all secondary metabolites that were tested in the plant samples. Results for the presence of alkaloids, saponins, coumarins, terpenoids, flavonoids and cardiac glycosides were positive while, Anthraquinones and Phlobatannins were absent in *S. mukorossi* and *A. concinna*. Figure **1** shows the average metabolite concentrations g/100g in the *Acacia* plant samples showed highest flavonoid content up to 9.5± 0.20 and lowest Alkaloid content upto 0.51± 0.04. *S. mukorossi* reported highest concentration of coumarins up to 7.6± 0.15 and lowest values of alkaloids up to 0.56± 0.01. The standard deviations were relatively low indicating the equal distribution of data across the means and uniformity in the results. The significant differences in the results of remaining phytochemicals within plants and between plants may be because of ecological factors such as climate, temperature, moisture, humidity, salinity, and water scarcity[22]. Similarly, amount of moisture in the environment and rainfall rate may variate the phytochemical content in the plant sample and extracts[23].

Flavonoids are the potential radical scavenging secondary metabolites that causes significant reduction in oxidative stress in cell lines, to generate immunity against diseases[24]. From the current study it can be seen that both plants extracts are rich in flavonoids content which concurs with the research outcomes of potential publications significantly[25,26]. The debatable point here is that both of the plant samples depicted least concentrations of alkaloids. A publication reported optimum levels of alkaloids in crude extracts of seeds which concurs with the present study[27]. Alkaloids own low palatability and considered as one of the noxious food compounds in the

hierarchy of the components, hence providing a validation for the safe consumption and application of fruit samples under study[28]. *Acacia* fruit extracts showed higher ratios of terpenoids as compared to the *Sapindus* showing more tendency to exhibit benefits for disease control and treatments. Terpenoids are structural components in dietary and medicinal plants[29]. The higher quantities of terpenoids in *Acacia* declare palatability of plant species. A research group examined different phytochemicals in plants samples[30]. The results exhibited the presence of terpenoids similar to current study which concurs with the standards set out by the previously reported scientific reviews. Coumarins are derivatives of pyro benzene and possess potential biological antimicrobial activity, and least toxicity was evidenced in *Sapindus* plant in the current study[31,32]. *Sapindus* plant has higher palatability as compared to other competent plants, and our study is in line with the previously declared use[19]. Anthraquinones and Phlobatannins are rarely occurring secondary metabolites across plant species[33,34], similar to current study where the metabolites were not evidenced. All of these outcomes indicated the selective proportions of metabolites in these plants and it can be a milestone in promoting selective extraction of these phytochemicals for further purposes.

Antioxidant Assays

Total Antioxidant Assay (TAOA)

Quantitative analysis of extracts was done by TAOA standard protocol. Results were compared with α-Tocopherol (Standard), 0.513 and 0.476 BHT, which validated that the sample qualities were quite similar to the said standards (α-Tocopherol: 0.513, BHT: 0.476, Blank: 0.026) adsorption at 695nm.

Among all solvents, the value for distilled water added *S. mukorossi* Gaertn. sample was 0.476 ± 0.096^{ab} being same as the value of standard chemical BHT, 0.476. Hence, it can be replaced by standard chemical.

In the case of *A. concinna*, distilled water indicated most extreme value i.e. 0.346 ± 0.050a while petroleum ether displayed least antioxidant agent value i.e. 0.263 ± 0.025a (Fig. **2**).

All of the values are an average of three replicates in which ± is denoted standard deviation at 0.5% significance level ($p \leq 0.05$)

Antioxidant Analysis through Radical Scavenging Activity of DPPH

Results were being equated with standard antioxidant available i.e (BHT, 0.190 and α-Tocopherol, 0.095, Blank, 0.03) absorption at 517nm. Among all the solvent extracts estimated, only few showed in line results to the standard values. Therefore, the closer solvents are recommended to be utilized as standards. Chloroform extract of *S. mukorossi* Gaertn. showed value 0.196 ± 0.029^{b} which is close to the standard BHT, 0.190 proving to be alternative to the standards (Fig **3**).

All of the values are an average of three replicates in which ± is denoting standard deviation at 0.5% significance level ($p \leq 0.05$).

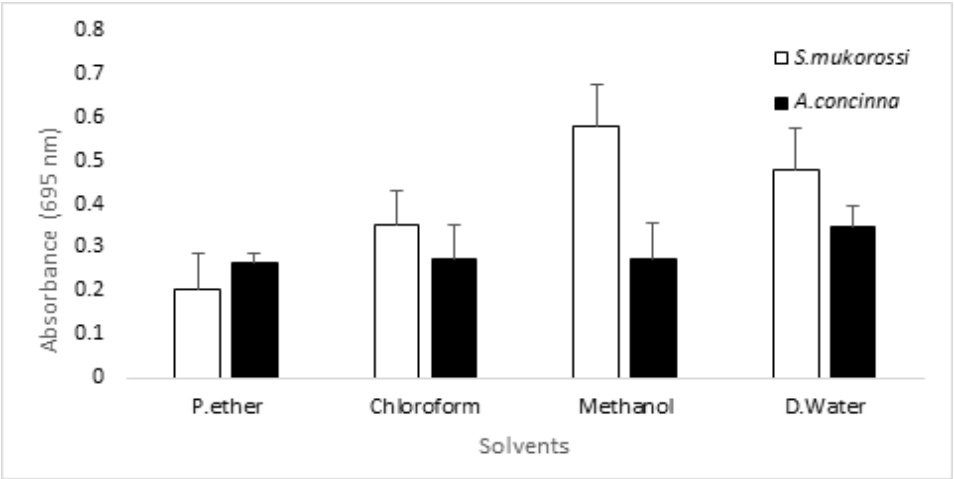


Figure 2. Antioxidant activity in plant extracts by TAOA adsorption at 695nm (Left: *S. mukorossi*; R.ight: *A. concinna*).

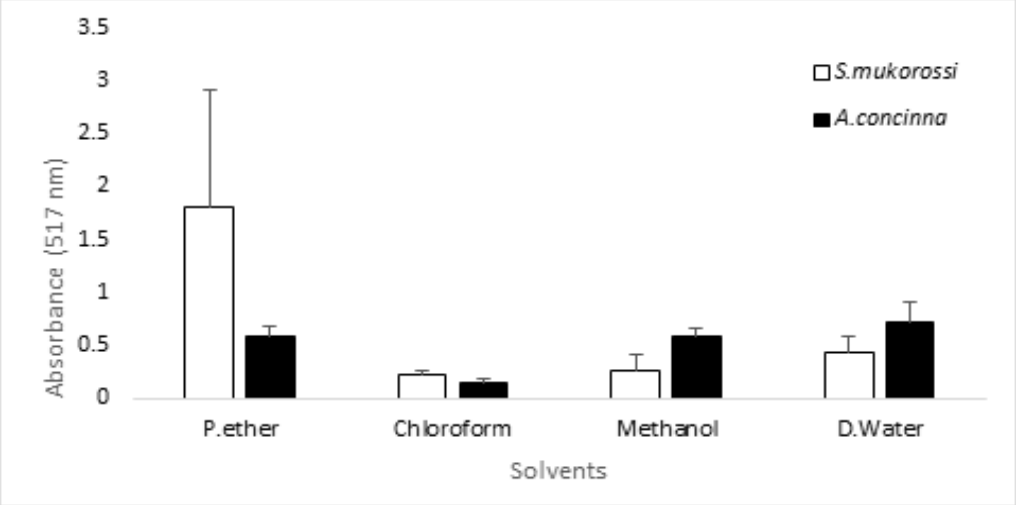


Figure 3. Antioxidant activity of plant extracts by DPPH analysis absorption at 517nm (Left: *S. mukorossi* Right: *A. concinna*).

Figures **2** and **3** reveal that antioxidant values of plants extracts concur to that of standards taken for the study. Total antioxidant activity and DPPH method values ranged from 0.203 to 0.576 at 695nm wavelength while 0.145 to 2.346 at 517nm wavelength which falls in line with comparative standard outcomes of previous research outcomes[35]. The generated outcomes from the assay displayed that plant extracts can scavenge the present radical up to a certain limit. A research group investigated the antioxidant potential by means of total antioxidant assay and free radical scavenging activity similar to the current study[36]. In our investigation methanolic extracts of *S. mukorossi* Gaertn. displayed maximum antioxidant value such as 0.576 ± 0.096^{a}. Whereas, petroleum ether extracts had lowermost value i.e. 0.203 ± 0.085^{c} at 695nm wavelength. Similarly, distilled water concentration 0.476 ± 0.096^{ab} which concurs with the value of standard BHT 0.476. At 519nm wavelength, *S. mukorossi* Gaertn. displayed highest antioxidant value in dis. Water extract as 0.456 ± 0.169^{b} and least with methanol such as 0.176 ± 0.035$^{b.}$ On the other hand, Chloroform extract value was found as 0.196 ± 0.029^{b} being very close to that of standard BHT, 0.190. Similarly, *A. concinna* (Willd.) DC. also indicated maximum result with distilled water as 0.723 ± 0.195^{a} and lowest with chloroform as 0.145 ± 0.045^{b}. Both outcomes lied closer to the standards utilized in the experimental approach that is BHT, 0.190. The similar values led to the advantage that these extracts can be utilized as standards[37].

Earlier reports showed that phytochemical compounds possess strong antioxidant activity for radical scavenging[38]. The radical scavenging ability leads to a reduction in oxidative stress and neutralizes the overall metabolism[39]. Plant origin antioxidant and oxidative stress reducing bio-products are on huge demand in the market due to least side effects and long-term benefits due to easy adjustment within the human body drug translocations[40]. This study revealed that massive potential of *Acacia concinna* and *Sapindus mukorossi* evidently constitutes optimum capacities of antioxidant mediated oxidative stress release as the recommended proportions for active cell lines. Most importantly, the polymer of Quinic acid is reported as the strongest scavenger by the neutral radical DPPH assay[41]. This polymer has been verified to be potent to that of BHT used as a positive control[42]. Hence, this study proves that it can be used as standard for further assays.

CONCLUSION

It is concluded that *S. mukorossi and A. concinna* proved active in the presence of secondary metabolites e.g. Alkaloids, Saponins, Terpenoids, Coumarins, Flavonoids and Cardiac glycosides, and exhibited the absence of Phlobotanins and Anthraquinones. Additionally, the antioxidant potential was perceived through Total Antioxidant Assay (TAOA) and DPPH Assays in which plant extracts especially distilled water extract of *S. mukorossi* Gaertn., Chloroform extract of *S. mukorossi* Gaertn. and Chloroform extract of A. *concinna* (Willd.) DC.

presented similar outcomes with standard antioxidants such as BHT and α-Tocopherol at two different wavelengths of 695nm and 517nm respectively. The results from current study showed *S. mukorossi* and *A. concinna* to be imperative and reliable for applications in chemical industry and pharmaceutical engineering.

ETHICAL APROVAL

The manuscript does not comprise any animal and human based studies hence no ethical approval was needed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING SOURCE

The research study was part of undergraduate thesis and did not receive any funding.

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LIST OF ABBREVIATIONS

%	Percentage
ANOVA	Analysis of Variance
BHT	butylated hydroxytoluene
C	Centigrade
DMSO	dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
g	gram
ml	mili liter
mM	mili molar
N	Normal
nm	Nano meter
SC%	Scavenging
TAOA	Total Antioxidant Assay
UV	ultraviolet

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