Original Research Article

Preliminary phytochemical profile, in vitro antioxidant and sun protective activities of Alhagi pseudalhagi and Elaeagnus angustifolia L.

ABSTRACT

Background and Objectives: *Alhagi pseudalhagi* and *Elaeagnus angustifolia* are among the medicinal plants used traditionally in Afghanistan. The aim of present work was to study *A. pseudalhagi* herbs and *E. angustifolia* leaves for their preliminary phytochemical profile, total phenolic content (TPC), total flavonoid content (TFC), *in vitro* antioxidant activity and sun protective factor (SPF).

Methods: Standard qualitative phytochemical tests were performed to detect various phytochemicals in the crude drugs. Prescribed methods were followed for determining TPC, TFC, and SPF of the plant extracts. To evaluate *in vitro* antioxidative potential, DPPH radical scavenging assay method was performed. All of the experiments were performed in three replications.

Results: Qualitative phytochemical tests revealed presence of carbohydrates, phenolics, flavonoids, sterols, tannins, and saponins in the plants extracts. The TPC of tested extracts ranged from 41.74 ± 1.22 to 203.76±2.07 mg gallic acid equivalent (GAE) / g dried extract, while the TFC ranged between 33.24±3.23 and 135.85 ±3.83 mg rutin equivalent (RUE) / g dried extract. The purified extract of *E. angustifolia* leaves contained higher TPC (203.76±2.07 mg GAE / g dried extract) and TFC (135.85±3.83 mg RUE / g dried extract). DPPH radicals scavenging capacity

and SPF of the tested extracts were positively correlated with their TPC and TFC. *Elaeagnus angustifolia* purified extract produced the lowest IC_{50} value of 4.5 and exhibited higher SPF of 29.73 ± 0.31 as calculated for 2mg/ml methanol solution of the extract.

Conclusion: overall result of present study showed that *E. angustifolia* leaves purified extract was rich in phenolics and flavonoids, and showed high SPF value, hence could be highly recommended to be used in development of herbal sunscreen formulations.

Keywords: *Alhagi pseudalhagi*, *Elaeagnus angustifolia*, total phenolic content, total flavonoid content, antioxidant, Sun protective factor

1. INTRODUCTION

Medicinal plants (MPs) have been used by mankind as curing agents since immemorial time, and still they are known as natural remedies for divers array of maladies. According to WHO, about 80% of world population still rely on traditional herbal medicines in case of their primary health care needs [1,2]. Herbal extracts owing to their complex compositions exhibit different biological and pharmacological effects; such as anti-inflammatory, antioxidant, antiapoptotic immunomodulatory, sun blocking, anti-cancer activities, and so on. Different botanical extracts are reported for their free radical scavenging capacity, anti-photo aging effects, and their role in treatment of different skin diseases and improvement of skin appearance [3-6].

Excessive solar UV radiations (UVR) that cause formation of excess Reactive Oxygen Species (ROS), are known to be responsible for various skin damages such as; sunburn, skin pigmentation, premature aging, photo carcinogenesis, and skin cancers [7-9].

Applying topical sunscreens is widely encouraged in order to protect skin from the adverse consequences of solar UVR. An effective sunscreen besides containing active screening agents, should contain antioxidants to scavenge ROS and to efficiently prevent skin from photo aging and skin cancers. Plants' antioxidants e.g. phenolic compounds and flavonoids are suitable candidates to be incorporated in sunscreen formulations [10]. Currently, plants as well as marine organisms are considered to be valuable sources of potential anti-photoaging and photoprotecive agents for development of cosmeceutical and topical pharmaceutical formulations [11].

Alhagi pseudalhagi (Syn. *A. maurorum*) belongs to family Fabaceae, is a small thorny shrub with stiff and spiny stem and branches. The plant is called *shuthur khar* in Afghanistan and is used in traditional medicine of the country for treatment of kidney disease, urinary tract infections, gonorrhea, bleeding, wounds, hypotension, and

gastrointestinal disorders [12]. *Alhagi pseudalhagi* is reported to be rich in several bioactive compounds e.g. flavonoids, flavone glycosides, alhagidin, alhagitin, proanthocynidins, triterpenes, tannins, and phenolic compounds [13, 14].

Elaeagnus angustifolia L**.** belongs to family Elaeagnaceae, is a small to medium sized tree or shrub, 2 – 5 m tall and grow abundantly in different parts of Afghanistan [15]. *Elaeagnus angustifolia* is used in traditional medicines as antipyretic, diuretic, tonic, antidiarrheal, anti-inflammatory, analgesic, and as a medication against kidney stones [16]. Different parts of the plant including its leaves are rich in various phytochemicals such as; polysaccharides, sitosteroles, glycosides, terpenoides, coumarins, saponins, carotenoids, vitamins, tannins, phenolics and flavonoid compounds that act as natural antioxidants [17-19].

Unfortunately, because of the long-lasting wars and other political issues, study of MPs grow in Afghanistan is neglected, and hence, publications regarding phytochemical profile and biological activity of Afghani MPs are very scarce. Considering worldwide interests into research on MPs and the huge demand of natural sunscreens, the main objectives of present research were to evaluate the preliminary phytochemical profile, total phenolic content (TPC), total flavonoid content (TFC), *in vitro* antioxidant potential, and sun protective factor (SPF) values of *A. pseudalhagi* herb and *E. angustifolia* leaves collected from Afghanistan.

2. MATERIAL AND METHODS

2.1 Plant materials

Elaeagnus angustifolia leaves were collected from Paghman district of Kabul on 7th of June, 2017, and *A. pseudalhagi* herb was collected at blooming time from Kabul University campus on 2nd July, 2017. Collected materials were botanically authenticated by Prof. Mohammad Nasim sediqi, Head of Pharmacognosy department, Faculty of Pharmacy, Kabul University. Collected materials were shade dried and after being coarsely powdered, were separately packed in polyethylene bags for further use. Voucher specimens of the collected plants were deposited in herbarium of faculty of Pharmacy, Kabul University, for further use as references.

2.2 Preparation of plants extracts

Conventional extraction method using soxhlet extractor was used in preparation of the crude extracts. 20 g of the dried and powdered *A. pseudalhagi* herb and *E. angustifolia* leaves were separately extracted with 70% methanol

(MeOH), at 70 ^{o}C, until the marc was exhausted. The extracts were filtered through Watmann No. 1, and then were concentrated under vacuum, at 40 ^{o}C. Concentrated extracts were further dried in oven at 60 ^{o}C, until getting their constant weights (complete drying). The dried *A. pseudalhagi* crude extract (APCE) and *E. angustifolia* crude extract (EACE) were kept in refrigerator at 4 ^{o}C for further uses.

2.3 Purification of extracts

To purify the extracts from chlorophyll and other inert lipophilic compounds, the method described by Jarzycka *et al.* with slight modifications were followed [20]. Accurately weighed amounts of both APCE and EACE were first dissolved in 70% MeOH, then extracted 4 times with equal amounts of its volume petroleum ether. The MeOH fractions were dried at 60 ^{o}C in drying oven. The dried extracts were then separately dissolved in hot distilled water, followed by addition of ascorbic acid (0.5 mg/g dried extract) to the mixture. Each of the mixture was left in refrigerator for 24 hours, and then, was extracted five times with diethyl ether. The last aqueous fractions were further extracted five times with ethyl acetate. In both cases, the ethyl acetate fraction was evaporated to obtain the dried *A. pseudalhagi* purified extract (APPE) and *E. angustifolia* purified extract (EAPE). APPE and EAPE were used for further studies. The yield percentages of the crude and purified extracts were calculated and shown in Table1.

2.4 Preliminary phytochemical screening

Methanol solutions (2 mg/ml MeOH 70%) of the extracts, e.g. APCE, EACE, APPE, and EAPE were separately prepared and subjected to preliminary phytochemical screening to detect diverse categories of phytochemicals in the extracts. Standard procedures and methods previously described were followed [21-23].

2.4.1 Detection of carbohydrates

2.4.1.1 Molisch's test: About 3 ml of test solution (TS) was mixed with 3 drops of Molisch's reagent (alpha-naphthol 20% in ethyl alcohol) in a clean test-tube. Then about 2 ml of conc. H_2SO_4 was carefully added along the side of the test-tube. Formation of a reddish violet or dark purple colour ring at the junction of the two layers indicated presence of carbohydrates.

2.4.1.2 Resorcinol test: A few small crystals of resorcinol were dissolved in about 2 ml of TS in a test-tube. Then an equal volume of conc. HCl was added to the test-tube, followed by warming on water-bath for a few minutes. Formation of a rose colour indicates presences of ketoses.

2.4.2 Detection of alkaloids

2.4.2.1 Dragendorff's test: three drops of Dragendorff's reagent (potassium Iodide + bismuth nitrate) were added on 2 ml of TS. Formation of an orange red/brown colour precipitate indicates presence of alkaloids.

2.4.2.2 Hagers' test: Few drops of Hagers' reagent (saturated picric acid solution) were added on 2 ml of TS. Formation of a yellow precipitate reveals a positive result.

2.4.2.3 Mayer's test: Few (2 - 3) drops of Mayer's reagent (solution of Potassium mercuric iodide) were added on 2 ml of TS. Formation of a white, pale yellow or cream precipitate indicates presence of alkaloids.

2.4.2.4 Wagner's test: About 2 – 4 drops of Wagner's reagent (Iodine solution) were added on 2 ml of TS in a test-tube. Formation of a brown or reddish-brown precipitate indicates a positive result.

2.4.3 Detection of glycosides

2.4.3.1 Legal's test: About 2 ml of TS were treated with 2 ml of sodium nitroprusside 2% solution in pyridine, and few drops of 20% sodium hydroxide in MeOH. The mixture was observed for formation of a pink to deep blood red colour.

2.4.3.2 Keller-Kiliani test: About 3 ml of TS were mixed with 2 ml glacial acetic acid and 1 ml ferric chloride (5%) solution. The mixture was heated on water-bath, then cooled and transferred to a test-tube containing 2 ml of concentrated H_2SO_4, and observed for formation of a reddish-brown colour at the junction of the liquids, and gradually the acetic acid layer turns blue.

2.4.4 Detection of anthraquinone glycosides

2.4.4.1 Borntrager test: Few mg of the test extract was treated with 5 ml HCl 0.5 N, then it was mixed with 5 ml chloroform for 15 minutes in a separatory funnel. Then, the mixture was placed aside for being separated into two phases. The chloroform phase was taken in a clean test-tube, followed by addition of 2 ml of 10% ammonia solution. The mixture was shaken well, then it was stand till the two phases were separated. Appearance of a pink or reddish colour in the aqueous phase indicated a positive result.

2.4.5 Detection of phenols

2.4.5.1 Ferric chloride test: About 2 ml of TS was placed in a clean test-tube, followed by addition of 2 – 3 drops of 1% ferric chloride solution. Formation of an intense greenish-black colour indicates presence of phenolic compounds.

2.4.6 Detection of flavonoids

2.4.6.1 Alkali reagent test: About 2 ml of diluted TS was placed in a test-tube. A few drops of NaOH 1N solution were added drop-wise in the test-tube. Increasing intensity of the yellow colour produced in the test-tube that would be colorless by addition of few drops of dilute HCl, indicates presence of flavonoids.

2.4.6.2 Shinoda/Pew test: A small amount of magnesium turnings was added into a test-tube containing about 3 ml of the TS. After addition of 3 drops of concentrated HCl in the test-tube, it was kept aside for completion of the reaction. After a while it was observed for formation of a reddish-pink or rose colour for a positive result.

2.4.7 Detection of tannins

2.4.7.1 Gelatin test: About 2 ml of TS was treated with 2 ml aqueous solution of 1% gelatin containing 10% sodium chloride. Formation of a white buff colour precipitate (a milky colour) indicates presence of tannins in the test sample.

2.4.8 Detection of saponins

2.4.8.1 Foam test: One ml of the TS after being diluted with 2 ml distilled water in a test-tube, was vigorously shaken for 15 minutes. Formation of persistent foam reveals presence of saponins in the test sample.

2.4.8.2 Fontan-kendale test: To differentiate between triterpenic and steroidic saponins, 1 ml of the TS was placed into two clean test-tubes (numbered 1 and 2), separately. Then, 5 ml of NaOH 0.1 N and 5 ml of HCl 0.1 N were mixed with the contents of test-tube No. 1 and No. 2, respectively. Both of the test-tubes were simultaneously shaken well, and were observed for the amount and stability of the foam produced in both test-tubes. Production of a persistent foam in test-tube No. 1 (containing mixture of test sample and diluted alkali), than in test-tube No 2 (containing mixture of test sample and diluted HCl) indicates presence of steroidic saponins in the sample.

2.4.9 Detection of sterols

A small amount of the dried extract was washed with petroleum ether and then with acetone. The residue was extracted with small amount (10 ml) of chloroform and the later was tested for detection of steroids.

2.4.9.1 Liebermann – Burchard's test: About 5 ml of the chloroform extract of the sample was placed in a test-tube, followed by addition of few drops of acetic anhydride. Then 2 ml of conc. H_2SO_4 were carefully added from the side of the test-tube. Formation of a brown to blood red colour at the junction of two phases indicates a positive result.

2.4.9.2 Salkowski Reaction: About 5 ml of the chloroform extract was placed in a test-tube, followed by carefully addition of about 2 ml of conc. H_2SO_4 from the side of test-tube. Formation of yellow-red or brown colour ring at the junction, which turns red after a short time, indicates presence of sterols in the sample.

Results of phytochemical screening of the tested extracts are presented in Table 2.

2.5 Determination of total polyphenolic content (TPC)

Total phenolic content of APCE, APPE, EACE and EAPE was determined as per Folin-Ciocalteu spectrophotometric method previously described [24, 25].

Briefly; 1 ml of extract MeOH solution having concentration of 300 µg/ml, was mixed with 1 ml Folin-Ciocalteu's Reagent (FCR) diluted (1/10) with distilled water in a clean test-tube. The mixture was shaken well, and placed at room ambience for 6 minutes, followed by addition of 3 ml Na_2CO_3 7.5 % aqueous solution. Blank was concurrently prepared as per the same procedure by substituting the extract with distilled water. Aliquots of gallic acid (GA) at concentrations of 5, 10, 20, 30, 40, and 50 µg/ml in MeOH, were used for establishing a standard calibration curve. All of the mixtures were shaken well, and incubated for 60 minutes at room ambience, subsequently centrifuged at 3000 rpm for 10 minutes. The supernatants were checked for their absorbances at 765 nm wavelength against the blank, using a spectrophotometer (Shimadzu UVmini 1240). TPC of the tested samples was calculated by use of the regression formula produced in GA calibration curve, and were expressed as mg GAE/g dried extract, as shown in Table 3.

2.6 Determination of total flavonoid content (TFC)

The APCE, APPE, EACE, and EAPE were screened for their TFC values, applying slightly modified Aluminum trichloride colorimetric method previously described [26, 27]. Briefly; $AlCl_3$ solution 2% (w/v) in MeOH was freshly prepared as reagent solution. Two ml of sample extract MeOH solution (300 µg/ml) were well mixed with 2 ml of the reagent in clean test-tubes. Two ml of Rutin (RU) aliquots at concentration range of 5 – 50 µg/ml were used for establishing a standard calibration curve. All of the mixtures were shaken well, and incubated at room ambience for 30 minutes in the dark for reaction completion. Then, absorbance of the mixtures was spectrophotometrically measured at 415 nm wavelength against the blank sample consisting of the mixture of 2 ml of sample/standard solution with 2 ml MeOH without $AlCl_3$. The TFC values were calculated based on the regression formula produced from RU standard curve, and were expressed as mg RUE/g dried extract, as shown in Table4.

2.7 Determination of *in vitro* antioxidant activity

For determination of *in vitro* antioxidant activity of tested extracts, DPPH assay method was used, as previously described [26, 28]. Accurately, 4 ml of the test extracts serial dilutions in MeOH having concentrations of 1.25, 2.5, 5, 10, 15, 20, and 25 µg/ml were separately mixed with 0.5 ml DPPH solution (0.5 mM) in MeOH. Likewise, 4 ml of serial dilutions (0.25, 0.50, 0.75, 1.00, 1.5, and 2.0 µg/ml) of ascorbic acid in MeOH were mixed with 0.5 ml DPPH solution (0.5 mM) in MeOH, to establish a standard calibration curve. As a positive control, 4 ml of pure MeOH was mixed with 0.5 ml DPPH solution (0.5 mM). All of the resultant mixtures were thoroughly shaken, and incubated in the dark at room temperature for 30 min for reaction completion. Then, absorbance of the mixtures were screened at 517 nm wavelength against methanol as blank, using a UV-spectrophotometer (Shimadzu UVmini 1240). The % inhibition for both of ascorbic acid and the tested extracts were calculated as shown in Fig. 1, and Fig. 2.

2.8 Determination of Sun protective factor (SPF)

The SPF is most commonly used parameter for expression of sun protective capacity of sunscreens [10, 29]. SPF is UV energy required to produce a minimal erythema dose (MED) on protected skin, divided by the UV energy required to produce a MED on unprotected skin. SPF is calculated using the following formula:

$$SPF = \frac{Minimal\ erythema\ dose\ in\ sunscreen\ protected\ skin}{Minimal\ eryhtma\ dose\ in\ nonsunscreen\ protected\ skin}$$

MED is the lowest time interval or dosage of UV light irradiation, required to produce a minimal, perceptible erythema or reddening on unprotected skin [30, 31].

SPF of tested extracts were determined by *in vitro* spectrophotometric method based on measuring transmittance of the extracts diluted solutions in MeOH. Transmittance of the extracts dilutions were measured in the range of 292.5 to 337.5 nm, at five nanometers intervals. SPF of extracts were calculated using the following formula adopted from pervious works [32, 33].

$$SPF = \frac{\sum_{292.5}^{337.5} E\ (\lambda) \times \varepsilon(\lambda)}{\sum_{292.5}^{337.5} E(\lambda) \times \varepsilon(\lambda) \times T(\lambda)}$$

Where E (λ) is the spectral irradiance of terrestrial sunlight; and ε (λ) is the erythema action spectrum at λ, and T (λ) is the measured transmittance at λ. The values of E (λ) and ε (λ) are constant, and derived from previous works. To establish a standard calibration curve, SPF of five different concentrations of the test extracts was

measured and the related graph was plotted. SPF values for tested extracts MeOH solution (2 mg/ml) were calculated using regression formula obtained from the plotted standard curve, and the results are shown in Table 5.

2.9 Statistical Analysis

All of the experiments were performed in triplicates (n=3) and values were expressed as Mean±SD. Statistical analyses were performed using Ms. Excel 2016 package. One-way analysis of variance (ANOVA) and Student t-test were used to assess the differences between different variables. All analyses were performed at the 5% significant level ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 Yield percentages of the extracts

The yield percentage of the extracts for *A. pseudalhagi* herb and *E. angustifolia* leaves (Table1.).

Table 1. Data showing the extractive % (w/w) of the plants

S. No.	Extract type	% extractive Mean±SD*
1	APCE	31.834±1.64
2	EACE	35.51±1.62
3	APPE	5.308±0.10
4	EAPE	4.475±0.08

** Mean ± Standard Deviation of the three experiments (n=3),*
APCE; A. pseudalhagi herb crude extract, APPE; A. pseudalhagi processed extract,
EACE; E. angustifolia leaves crude extract, EAPE; E. angustifolia processed extract

As shown in Table 1, the highest yield is recorded for EACE (35.51±1.62 %), followed by APCE (31.834±1.64 %), while yields % for APPE and EAPE were recorded to be 5.308±0.10 and 4.475±0.08, respectively.

As data show, the percentage yields of the purified extracts are lower than that of the crude extracts, that indicates most of extra materials such as chlorophyll and other lipophilic substances are removed during washing of the crude extract by petroleum ether. Such purified extracts are proved to pose more potent biological efficacy because of removal of the inert compounds thereof.

3.2 Phytochemical screening of the extracts

Phytochemicals present in the tested extracts are shown in Table 2.

Table 2. Phytochemicals detected in the tested extracts

S. No.	Phytochemicals	APCE	APPE	EACE	EAPE

01	**Carbohydrates**				
	Molisch's test	++	+	++	+
	Resorcinol test	+++	+	++	+
02	**Phenols**				
	FeCl3 Test	++	+++	++	+++
03	**Flavonoids**				
	Alkali R. Test	+	+++	++	+
	Shinoda Test	+	++	++	+
04	**Tannin**				
	Gelatin test	++	-	++	-
05	**Saponin**				
	Foam test	+	++	+	-
	Fontan-Keldale test	triterpenic	triterpenic	steroidic	-
06	**Sterols**				
	Liebermann – B test	+	+	+	+
	Salkowski test	+	+	+	+

APCE; A. pseudalhagi herb crude extract, APPE; A. pseudalhagi processed extract, EACE; E. angustifolia leaves crude extract, EAPE; E. angustifolia processed extract, '+' Present, '++'moderately present, '+++'intensely present, '-' absent

As shown in Table 2, different classes of phytochemicals such as; carbohydrates, flavonoids, phenolic compounds and saponins were found to be present in all tested extracts. Based on qualitative phytochemical tests, *A. pseudalhagi* herb contains triterpenic saponins while *E. angustifolia* leaves contain steroidic saponins. However, further works and quantitative tests are required for accurate determination of the phytochemicals present in the tested extracts. Based on available literature, presence of diverse array of secondary metabolites are reported in *Alhagi* species including *A. pseudalhagi* [14, 34, 35]. Similarly, *E. angustifolia* L. is reported to contain different compounds such as; alkaloids, flavonoids, polysaccharides, saponins, tannins, vitamins, and metal elements in different plant parts [17, 18, 36, 37].

3.3 Total phenolic content (TPC) of the extracts

In present study, extracts MeOH solution at (300 µg/ml) were screened for their TPC values, applying Folin-Ciocalteu method previously described [25, 38]. TPC of tested samples were calculated from the regression equation ($y=0.0201x + 0.024$, $R^2=0.995$) obtained from GA standard curve, and were expressed in terms of GA equivalent (mg of GAE/g of dried extract), as shown in Table 3.

Table 3. Data showing the TPC values of tested extracts

S. No.	Extract type	TPC values *(mg GAE/g dried extract)
1	APCE	71.14±3.10
2	APPE	80.43±3.69
3	EACE	82.64±2.57
4	EAPE	203.76±2.07

** The values are recorded as Mean±SD, (n=3)*

APCE; Alhagi pseudalhagi crude extract, APPE; A. pseudalhagi processed extract, EACE; Elaeagnus angustifolia crude extract, EAPE; E. angustifolia processed extract

As shown in Table 3., the order of TPC in tested extracts was highest for EAPE > EACE> APPE >APCE. Consequently, among tested extracts, both of the EAPE and EACE exhibited highest TPC values of 203.76±2.07 and 82.64±2.57 mg GAE/g dry extract, respectively. The data showed that purified extracts revealed higher TPC values than that of crude extracts. Plants phenols by virtue of their aromatic rings, show an intense absorption in the UV region of the spectrum [22]. Hence, they would serve as choice compounds to be incorporated in sunscreen formulations.

3.4 Total flavonoid content (TFC) of the extracts

Determination of TFC of botanicals is of high importance in predicting biological activities such as; antioxidative potential of tested crude drugs. In present study, TFC of tested extracts were calculated from the regression equation ($y=0.023x - 0.072$, $R^2=0.9996$) obtained from RU standard curve. The TFC values were expressed in terms of rutin equivalent (mg of RUE/g of dried extract), and shown in Table 4.

Table 4. Data showing TFC values of tested extracts

S. No.	**Extract type**	**TFC values *(mg RUE/g dried extract)**
1	APCE	59.61±3.40
2	APPE	121.06±3.37
3	EACE	33.24±3.23
4	EAPE	135.85±3.83

** The values are recorded as Mean±SD, (n=3)*

APCE; Alhagi pseudalhagi crude extract, APPE; A. pseudalhagi processed extract, EACE; Elaeagnus angustifolia crude extract, EAPE; E. angustifolia processed extract

As shown in Table 4., the order of TFC of tested extracts was highest for EAPE > APPE> APCE >EACE, respectively. TFC of the tested extracts varied from 33.24±3.23 to 135.85±3.83 mg RUE/g dried extract. The highest flavonoids content (135.85±3.83 mg RUE/g extract) was recorded for EAPE.

3.5 *In vitro* antioxidant activity of the plant extracts

Evaluation of antioxidative potential of botanicals serves as a choice approach to judge their pharmaceutical as well as pharmacological values. In present work, sample extracts at concentration range of 2.5 – 25 µg/ml in MeOH were evaluated for their DPPH free radical scavenging activity. Ascorbic acid serial dilutions at concentration range of 0.25 – 2 µg/ml in MeOH were used as standard antioxidant compound. Radical scavenging activity (RSA) of the test extracts as well as standard ascorbic acid was calculated as percentage inhibition (% inhibition) of DPPH free radicals, applying the following formula:

$$\% \text{ inhibition} = 100 \times [(Ac - As) / Ac]$$

Where, **A*c*** is the absorbance of control, and **A*s*** is absorbance of sample/standard

Antioxidative potential of the standard ascorbic acid and tested extracts were expressed as inhibition concentration of 50% DPPH free radicals (IC_{50}), which were calculated from their plotted graph of % inhibition. Fig. 1, represents the % inhibition of DPPH free radicals by the tested extracts.

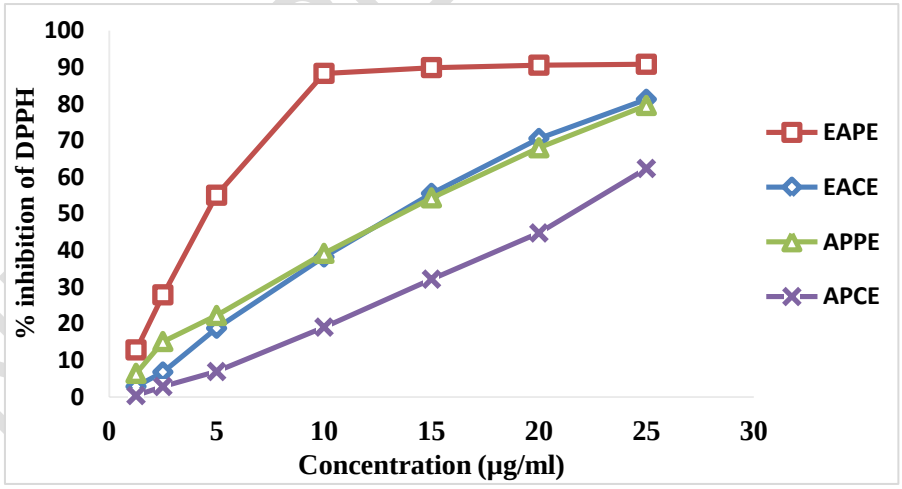

Fig. 1. Graph showing % inhibition activity of tested extracts in DPPH assay: *EAPE; E. angustifolia processed extract, EACE; E. angustifolia leaves crude extract, APPE; A. pseudalhagi processed extract, and APCE; A. pseudalhagi herb crude extract*

As shown in Fig. 1, the RSA of the tested samples was dose-dependent. That is to say, the % inhibition of DPPH free radicals were increasing by increase in concentration of test extracts solutions used in the assay.

The antioxidant potentials of standard ascorbic acid and tested extracts were expressed as their IC_{50} values. IC_{50} actually refers to the concentration (µg/ml) of standard or sample extracts required for inhibition of 50% DPPH radicals used in the assay. Lower IC_{50} value of a test sample indicates higher RSA and higher antioxidant activity of the same sample [26, 27, 39].

In vitro antioxidative potential of tested extracts were expressed as their IC_{50} values which were calculated from their plotted graphs of % inhibition vs. concentrations (Fig. 1.). Fig. 2 represents the calculated IC_{50} values for both ascorbic acid and the tested extracts.

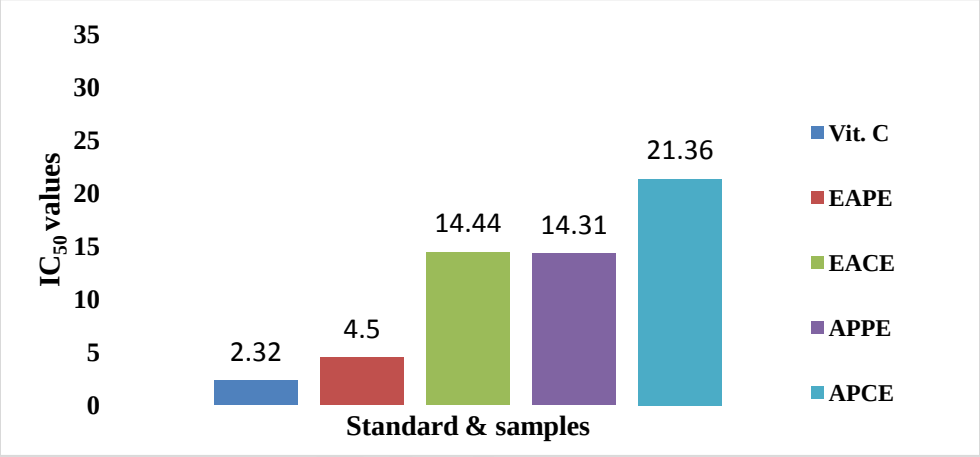

Fig. 2. Graph showing the IC_{50} values for the standard and tested extracts: *Vit. C; vitamin C, EAPE; E. angustifolia processed extract, EACE; E. angustifolia leaves crude extract, APPE; A. pseudalhagi processed extract, and APCE; A. pseudalhagi herb crude extract*

As shown in Fig. 2, IC_{50} for ascorbic acid is 2.32 µg/ml, while IC_{50} of the tested extracts are in range of 4.5 – 21.36 µg/ml. EAPE exerts the highest free RSA (IC_{50} = 4.5), while RSA of the remaining extracts decreased in order of APPE>EACE>APCE with IC_{50} values of 14.31<14.36<21.36, orderly.

In DPPH assay, the stronger activity of EAPE could be attributed to its purity from other inert phytochemicals such as chlorophyll, etc. and its richness in phenolics compounds. Therefore, a direct correlation exist between TPC and DPPH radical scavenging potential of EAPE. Our data were in compliance with those of many previous works which reported positive correlation between DPPH radical scavenging activities and TPC values of tested plant extracts [26, 40]. As per another study, high phenolic content and strong antioxidant properties of ethylacetate fraction of defatted methanolic extract of *Allium* species was reported [41]. In another study,

methanol extracts of cinnamon and cloves exhibited strong antioxidant properties owing to their high phenolic and flavonoids content [38]. Positive correlation between TPC and in vitro antioxidant potential of *Aloe vera* leaves is also reported [42].

3.6 Sun protective factor (SPF) of the extracts

SPF values of the tested extracts' MeOH solution (2mg/ml) are presented in Table 5.

Table 5. Data showing SPF value for the tested extracts 2mg/ml methanolic solutions

S. No.	Extract type	SPF* (Conc. 2 mg/ml)
1	APCE	5.47 ± 0.03
2	APPE	17.83 ± 0.88
3	EACE	10.69 ± 0.16
4	EAPE	29.73 ± 0.31

** Data were recorded as Mean±SD, (n=3).*

APCE; Alhagi pseudalhagi crude extract, APPE; A. pseudalhagi processed

As per the results, the SPF values were between 5.47 ± 0.03 and 29.73 ± 0.3. As data showed, both of EAPE and APPE exhibited higher SPF than EACE and APCE. This issue could be attributed to the purity of EAPE and APPE from excess of chlorophyll and other inert compounds. The higher amounts of both polyphenols and flavonoid compounds in EAPE and APPE, and elimination of their inert chemicals, are claimed to be the reasons for their increased UV absorption.

The *In vitro* spectrophotometric method used in present study, was based on measuring transmittance of diluted solution of the tested extracts. Since botanical extracts contain a wide range of phytochemicals such as; phenolic compounds, flavonoids, carotenoids, vitamin E, vitamin C and etc. hence these compounds are responsible for UV absorption and sun protective properties of botanicals [10, 29, 43].

In present study, positive correlations were found between DPPH radical scavenging capacity and SPF values of the tested extracts. Likewise, there was positive correlation between SPF and TPC, and also between SPF and TFC of the tested extracts. Our findings were in compliance with many previous works, which have reported that both phenolics and flavonoid compounds act as UV absorbing agents [10, 32, 44]. In addition, phenolics and flavonoids as strong antioxidant agents, play important roles in prevention of UV induced damage such as; photo aging and skin cancers [20, 45]. Recently, antioxidants present in botanicals and marine organisms are deemed to serve as compounds of choice for developing natural sunscreen formulations [10, 11].

4. CONCLUSION

Considering the huge demand for safe and effective plants derived UV-filters, *A. pseudalhagi* herb and *E. angustifolia* leaves were screened for their antioxidant potential and sun protective factor. Purified extract of *E. angustifolia* leaves being rich in phenolics and flavonoids, demonstrated strong DPPH radicle scavenging activity and a high SPF of 29.73 ± 0.31 for its MeOH solution (2mg/ml). Hopefully, *E. angustifolia* leaves could be recommended as natural source of the sun protective agents beneficial in developing potent herbal sunscreen formulations.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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