Research Article

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Suitable solvent and drying condition to enhance phenolics and extractive value *of Saussurea costus*

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ABSTRACT

Plants are rich source of medicinally important compounds such as phenolics compounds and flavanoid used as antioxidant and have chemo-preventive role against the risk of oxidative stress-related diseases. *Saussurea costus* (Falc.) Lipsch. (Asteraceae) have medicinal properties, such as anti-inflammatory, antioxidant, hepatoprotective, anti-ulcer, anticancer, immunomodulatory and pesticidal activities. Phytochemical investigation was carried out with hexane, chloroform, methanol and ethanol extract. six extracting solvents, methanol, ethanol, aqueous methanol (80% v/v), aqueous ethanol (80% v/v), aqueous methanol (70% v/v) and aqueous ethanol (70% v/v) were applied for their efficacy to extract antioxidants from air-dried, sun-dried and oven-dried roots of *Saussurea costus*. There was a significant difference ($P < 0.05$) in the extracting ability of each of the solvents. The aqueous solvents were found superior in their ability to extract the antioxidants and aqueous ethanol was reported more efficient than aqueous methanol. Highest and lowest extractive value and antioxidants compounds were found from respectively Oven dried (40°C) and air dried (ambient, approx 25°C) *Saussurea costus* roots. There was excellent correlation between extraction yield, antioxidant activity and total phenolic content.

Keywords:extraction yield, phytochemical screening, phenolics, solvent, drying method.

INTRODUCTION

***S**aussurea costus* (Falc.) Lipschitz (Asteraceae) is a well known and important medicinal plant commonly known as costus, kuth, kushta, kust, muxiang, patchak, quang mu xiang is widely used in Ayurveda and Chinese systems of medicine for the treatment of various ailments, viz. asthma, inflammatory diseases, ulcer and stomach problems. Different pharmacological experiments in a number of *in vitro* and *in vivo* models have convincingly demonstrated the ability of *Saussurea costus* to exhibit anti-inflammatory, anti-ulcer, and anticancer and hepatoprotective activities [1].

The roots contain odorous principles composed of two liquid resins, an alkaloid, a solid resin, salt of valeric acid, an astringet principal and ash which contain manganese. The oil of root was found to have the following approximate composition:- Camphene 0.04%, phellandren 0.4%, terpen alcohol 0.2%, a-costen 6.0%, aplotaxene 20.0%, costol 7.0%, di-hydrocostus lactone 15.0% costus lactone 10.0%, costic acid 14.0%. Active principal of the root are (a) an essential oil of a strong aromatic penetrating and fragrance odour 1.5%. (b) aglucoside and (c) an alkaloid Saussurine 0.05%. Kuthroots contain resinoids (6%), and essential oil (1.5%), alkaloid (0.05%) inulin (18%), saussurea lactone (20-25%), a fixed oil and minor constituents like tannin and sugars [2,3].

In present study, phytochemical analysisand extractive value, total phenolics and antioxidant activity of *Saussureacostus* roots were performed with different extracting solvent and drying condition.

MATERIAL AND METHOD

Experimental

Material and Reagent

Reference standard, Rutin and gallic acid were procured from M/s Natural remedies Pvt. Ltd. Bangalore, India. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

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Collection of powdered roots sample

Roots of *Saussurea costus* were collecte from wild source in Palampur, Himachal Pradesh, India in March, 2012. These roots were authenticated by Dr. Sayeed Ahmad, Faculty of Pharmacy, Jamia Hamdrad, New Delhi comparing with the specimen no. 34 available at Department of Pharmacognosy, Jamia Hamdard. The roots were dried at room temperature and powdered.

Drying of samples

The chopped Saussureacostus roots was divided into three portions (500 g each) to dry by different drying processes. One portion was air-dried (ambient conditions, 10 days), another portion was sun-dried (7 days) and a third portion was oven-dried at 40°C (3 days). All samples were ground and the material that passed through 80-mesh was used for extraction purposes.

Preparation of extracts

For quantitative analysis of phenolics and *in vtro* antioxidant activity, 100 gm powder root of *Saussurea costus* was extracted with solvent ethanol:water (80:20), ethanol:water (70:30), 100% ethanol, methanol:water (80:20), methanol:water (70:30) and 100% methanol. The extraction was performed by an orbital shaker for 24 hrs at room temperature and then the extracts filtered through filter paper (Whatman No. 1). The residue was re-extracted with a further two aliquots of fresh solvent following the same procedure. The combined extracts were evaporated to dryness using a rotary evaporator. The crude concentrated extract was weighed and stored at 4°C.

For phytochemical screening dried and ground plant material (1.0 kg) was successively extracted with hexane, chloroform, ethanol and methanol for 6 h each. The extracts were concentrated to dryness under reduced pressure. The obtained extracts were stored in a refrigerator at 4°C until use.

Phytochemical analysis

Preliminary phytochemical screening was perfomed. The presence of phytoconstituents such as Tannins, Saponins, Phenolic, Terpenoids, Steroids, Phytosterol Anthraquinone Glycosides and Flavanoid were confirmed by the following procedure [4].

Test for tannins

About 2 ml of filtered extract was taken in a test tube and 2 ml of ferric chloride added. The presence of blue-black coloured precipitate indicates the presence of tannins.

Test for saponins

To 0.5 ml of extract was added 5ml of distilled water in a test tube. The solution was shaken vigorously and observed for stable persistent forth.

Test for terpenoids (salkowski test)

To 0.5 ml each of the extract was added 2 ml of chloroform. Concentrated Sulphuric acid 3 ml was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for cardiac glycolsides

To 2 ml of extract 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in theacetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for anthraquinone

0.5 ml of the extract was boiled with 10 ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette in to another test tube observed for colour changes.

Test for flavonoids

Dilute ammonia 5 ml was added to the extract. Concentration sulphuric acid 1 ml was added. A yellow colouration that disappears on standing indicates the presence.

Test for steroids

To 1 ml extract 10 ml of chloroform was added. 10 ml of concentrated sulphuric acid was added carefully to form coloured layer. Upper layer turns red. Sulpuric acid layer forms yellow with green fluorescence, indicates the presence of steroids.

Test for phytosterol

1 ml of extract was dissolved in 10 ml of chloroform and 10 ml concentrated sulphuric acid along the side of the test tube. Brown ring indicates presence of phytosterol.

Test for phenolic

2 ml of extract 1 ml ferric chloride was added, a blue or green colour indicates presence of phenolic.

Determination of total phenolics content [5]

Preparation of sample

0.5 ml of extract solution (1mg/ml) was added to 5 ml of 10% Folin-Ciocalteu reagent and 4 ml of 1M Na_2CO_3 solution, mixed and allowed to stand for 15 minute in the dark. The absorbance of reaction mixtures were measured at 765 nm. The total phenolics content was expressed as mg Gallic acid equivalents / 100 g dry weight (d.w.) of the extract.

Preparation of standard

0.5 ml of standard dilution of gallic acid (10µg, 20 µg, 50 µg and 100 µg) was added to 5 ml of 10% Folin-Ciocalteu reagent and 4 ml of 1M Na_2CO_3 solution, mixed and allowed to stand for 15 minute in the dark. The absorbance of reaction mixtures were measured at 765 nm.

Determination of total flavonoid content [5]

Preparation of sample

0.5 ml of extract of solution (1mg/ml) was added to 1.5 ml methanol and mixed well. After that 0.1 ml of $AlCl_3$ (0. 1mg/ml) and 0.1 ml of 1M CH_3COONa reagents were added to above solution. This reaction mixture was added to 2.8 ml of distilled water, mixed and allows to stand for 30 minutes in dark. The absorbance of reaction mixtures were measured at 415 nm. The total flavonoid content was expressed as mg rutin equivalents / 100 g d.w. of the extract.

Preparation of Standard

0.5 ml of standard dilution of rutin (10µg, 20 µg, 50 µg and 100 µg) was taken and added to 1.5 ml methanol and mixed. After that 0.1 ml of $AlCl_3$ (0. 1mg/ml) and 0.1 ml of 1 M CH_3COONa reagents were added to

above solution. This reaction mixture was added to 2.8 ml of Distilled water, mixed and allows standing for 30 minutes in dark. The absorbance of reaction mixtures were measured at 415 nm.

Blank solutions

2 ml of methanol was added to 0.1 ml of $AlCl_3$ and 0.1 ml of CH_3COONa reagents and then added to 2.8 ml Distilled water and mixed.

Determination of antioxidant activity

DPPH free radical scavenging activity

0.1 mM solution of 1, 1- diphenyl-2-picryl-hydrazyl (DPPH·) in methanol was prepared and 1 mL of this solution was added to 3 mL of each alcohol extract at one concentration (500 µg/mL). Butylated hydroxytoluene (BHT) was used as a positive control. Discoloration was measured at 517 nm after incubation for 30 min. Measurements were taken at least in triplicate. The capacity to scavenge the DPPH radical was calculated using the following equation: DPPH scavenging effect (%) = [A*DPPH* − A*S* / A*DPPH*] x100 where, A*DPPH* is the absorbance of the DPPH solution and A*S* is the absorbance of the solution when the sample extract is added. The extract concentration providing 50% inhibition of radical-scavenging activity (IC_{50}) was calculated and expressed as mg/mL, d.w. [6].

Ferric reducing power determination

Alcohol extracts at concentration of 500 µg/mL were mixed with phosphate buffer (2.5 mL, 200 mM, pH 6.6) and 1% potassium ferricyanide (2.5 mL). Then the mixture was incubated at 50 °C for 20 min.2.5 mL of 10% trichloroacetic acid was added to above mixture and centrifuged at 10000 rpm for 10 min. The upper layer of the solution (5 mL) was mixed with distilled water (5 mL) and 0.1% ferric chloride (1 mL). The absorbance of the reaction mixture was measured at 700 nm. The final results were expressed as µg ascorbic acid equivalents / g based on dry weight of the extract [7].

Hydrogen peroxide scavenging activity

Solution of H_2O_2 (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). Extracts at concentration 50 µg/mL dissolved in 3.4 mL phosphate buffer were added to a H_2O_2 solution (600µL). The absorbance of the reaction mixture was recorded at 230 nm. BHT at concentration 50µg/mL used as positive control and percentage H_2O_2 scavenging effect was calculated as [A*Control*– A*Sample*/ A*Control*] x 100 where A *Control* is the absorbance of the control (blank, without extract), and A *Sample* is the absorbance in the presence of the sample extract. The extract concentration providing 50% of H_2O_2 scavenging activity (IC_{50}) was calculated and expressed as µg/mL based on sample dry weight [8].

RESULT AND DISCUSSION

Phytochemical analysis

The results of phytochemical screening of extracts revealed the presence of phenolic compounds, flavonoids, terpenoids, alkaloids, protein and cardiac glycosides in root extracts of *Saussurea costus* (Table 1). Particularly, methanol, ethanol and chloroform extracts of *Saussurea costus* were good sources of different classes of compounds. This indicates that these solvents are effective to isolate active biological compounds due to their high polarity. Flavonoids were detected in chloroform, acetone, ethanol and methanol extracts of root except petroleum ether extract. Flavonoids belong to the group of polyphenolic compounds and are typically known for health promoting properties such as antioxidant, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties [9]. They exist widely in the plant kingdom and displayed positive correlation between increased consumption of flavonoids and reduced risk of cardiovascular and cancer diseases [10]. Correspondingly, these extracts also tested positive for phenolic compounds. The phenolic compounds are aromatic secondary metabolites that impart colour, flavour and associated with health benefits such as reduced risk of heart and cardiovascular diseases [12, 13]. According to Aliyu*et al*.[13] phenolic compounds account for most of the antioxidant activities in plants. All the extracts of *Saussurea costus* except acetone extract have been detected for the presence of terpenoids, although saponins were altogether absent in allroot extracts. Terpenoids such as triterpenes, sesquiterpenes and diterpenes have been referred to as antibiotics, insecticidal, anthelmintic and antiseptic in pharmaceutical industry [14]. The alkaloid was observed in chloroform, Benzene, ethanol and ethanol extracts except pet ether extract. Alkaloids have been reported to possess analgesic, antispasmodic and bactericidal, antimalarial and analgesic activities [15].

Table 1: Preliminary phytochemical screening of different solvent extracts from roots of *Saussurea costus*

Constituent	Solvent extract				
	Hexane	Benzene	acetone	chloroform	methanol
Alkaloids	—	++	+	++	+
Carbohydrates	—	—	+	—	+
Glycosides	—	++	–	++	–
Phenolic compounds and tannins	—	+	+	++	+
Flavonoids	—	++	+	++	+
Proteins and free amino acids	—	—	–	—	–
Saponins	—	—	–	_+	–
Steroids	++	—	+	—	+
Terpenoids	++	_+	–	—	+

++ Present, - Absent

Yield of extraction

The aqueous solvents were superior in their ability to extract the antioxidants and highest extractive value (6.7 g/100 g dry weight) was found with aqueous ethanol from oven dried drug. Extraction with pure methanol offered the least yield (2.4 g/100 g dry weight) from air dried drug. Table 2 shows Effect of extracting solvents and drying processes on the extraction yield (g/100g dry weight) from *Saussureacostus* roots.

Table 2: Effect of extracting solvents and drying processes on the extraction yield (g/100g dry weight) from *Saussurea costus*

Extracting solvent	Extraction yield(g/100g, mean ± SD)		
	Air dried drug	Sun dried drug	Oven dried drug
100% methanol	2.4±0.265	3.3±0.342	4.5±0.342
80% v/v methanol	3.1±0.321	4.4±0.311	5.6±0.423
70% v/v methanol	3.8±0.453	4.9±0.454	5.9±0.234
100% ethanol	2.8±0.198	3.9±0.288	5.1±0.549
80% v/vethanol	3.4±0.546	4.8±0.355	6.2±0.268
70% v/v ethanol	4.4±0.276	5.3±0.398	6.7±0.399

*=average of three estimations at each level

These findings could be supported by other studies reported in the literature, where methanol and ethanol with some water content (typically 20 - 40%) have been found to be superior in extracting antioxidant compounds from a wide range of plants [16-18].

Total phenolics and flavanoid content in different extracts of roots of *Saussurea costus*

The total phenolic and flavanoid content of different extracts of *Saussurea costus* root as determined by Folin-Ciocalteu method are reported respectively as gallic acid and rutin equivalents. Among the six extracts, aqueous ethanol (70 % v/v) extract from oven dried drug showed the highest phenolics and flavanoid content respectively (65 µg/g and 130 µg/g). Figure 1 and 2 shows total phenolics and flavanoid content in different solvent extracts from oven dried, sun dried and air dried roots of *Saussurea costus*.

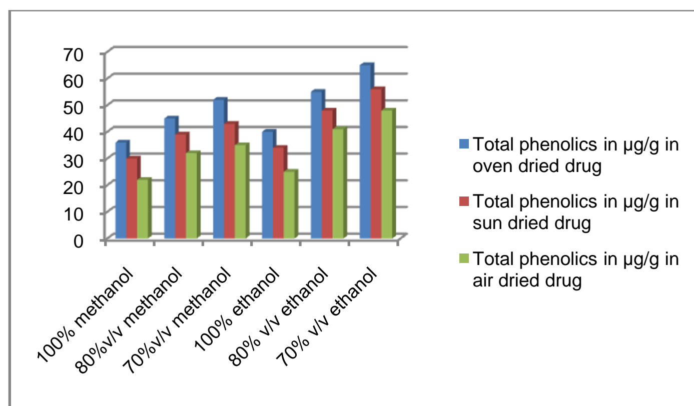

Figure 1: Quantitative analysis of total phenolics from powder roots of *Saussurea costus with* different extraction solvent and dried condition

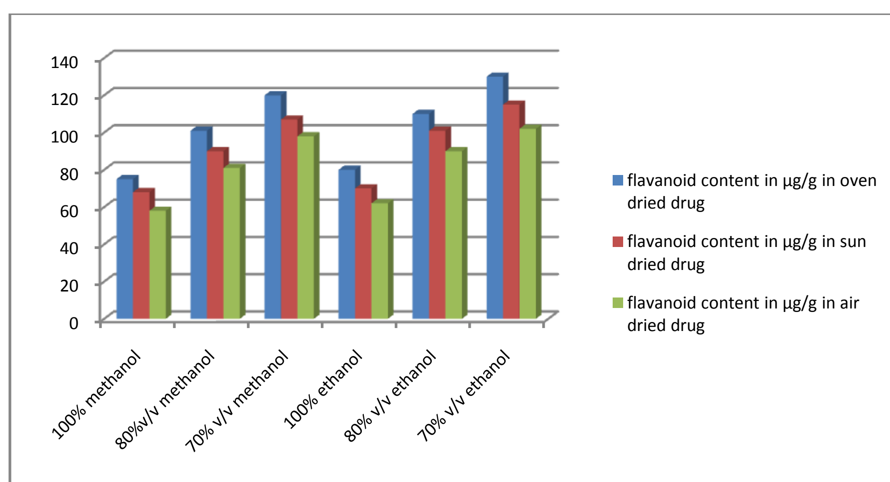

Figure 2: Quantitative analysis of flavanoid content from powder roots of *Saussurea costus with* different extraction solvent and dried condition

Evaluation of *in vitro* antioxidant assay

The high DPPH activity could be correlated with high phenolics content. Absorbance of DDPH radical decrease with high phenolic content. DDPH acts as a stable free radical in extract solution that easily accepts an electron or hydride radical and converted to a stable diamagnetic molecule. By reacting with suitable reducing agents DPPH radicals formed into the corresponding hydrazine.

Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. The generation of even low levels of H_2O_2 in biological systems may be important. Naturally-occurring iron complexes inside the cell believed to react with H_2O_2 *in vivo* to generate highly reactive hydroxyl radicals and this may be the origin of many of its toxic effects [19]. Hydrogen peroxide itself is not very reactive, but can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells [20]. Thus, removal of H_2O_2 is very important for protection of food systems. Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water.

The great amount of antioxidants in the extracts from *Saussurea costus* root would be resulted in the reduction of Fe3+ and Fe2+ by providing an electron. The amount of $Fe3^{+}$and $Fe2^{+}$ can be indicated by the Perl's blue colour appearance and determined by the absorbance at 700nm [21]. There is the significant correlation between the phenolic compounds and antioxidant activity [22]. Reducing power has mainly caused by the presence of the high phenolic compounds of the plant [23].

Highest DPPH free radical scavenging activity, ferric reducing power activity and hydrogen peroxide scavenging activity were found in aqueous ethanol (70 % ethanol v/v) from oven dried *Saussurea costus* roots. Table 3, 4 and 5 show DPPH free radical scavenging activity, ferric reducing power activity and hydrogen peroxide scavenging activity in different extracts from oven dried, sun dried and air dried *Saussureacostus* roots.

Table 3: Evaluation of *in vitro* antioxidant assay of different solvent extracts from oven dried *Saussurea costus* roots

Solvent extract	*DPPH scavenging activity (%, mean ± SD)	*IC_{50} values (mg/mL, d.w.) of DPPH scavenging activity	*Ferric reducing power(µg/g, dw)	*Hydrogen peroxide scavenging activity (%, mean ± SD)	*IC_{50} values (µg/mL, d.w.) of H_2O_2 scavenging activity
100% methanol	70.63 ± 0.176	0.513 ± 0.034	18.4 ± 0.163	64.63 ± 0.176	63.54 ± 0.143
80% v/v methanol	71.31 ± 2.685	0.601 ± 0.084	18.1 ± 0.182	65.31 ± 0.585	70.34 ± 0.353
70% v/v methanol	83.89 ± 2.224	0.711 ± 0.074	21.9 ± 0.193	72.89 ± 0.224	80.5 ± 0.273
100% ethanol	74.31 ± 0.585	0.654 ± 0.031	19.2 ± 0.113	66.63 ± 0.176	65.8 ± 0.393
80% ethanol	74.7 ± 0.463	0.649 ± 0.024	19.6 ± 0.124	66.32 ± 0.213	72.70 ± 0.463
70% ethanol	85.31 ± 2.685	0.890 ± 0.084	25.3 ± 0.182	75.31 ± 0.585	82.34 ± 0.353
Control(BHT)	76.31 ± 0.585	0.754 ± 0.031	20.2 ± 0.113	69.63 ± 0.176	67.8 ± 0.393

*=average of three estimations at each level

Table 4: Evaluation of *in vitro* antioxidant assay of different solvent extracts from sun dried *Saussurea costus* roots

Solvent extract	*DPPH scavenging activity (%, mean ± SD)	*IC_{50} values (mg/mL, d.w.) of DPPH scavenging activity	*Ferric reducing power (µg/g, dw)	*Hydrogen peroxide scavenging activity (%, mean ± SD)	*IC_{50} values (µg/mL, d.w.) of H_2O_2 scavenging activity
100% methanol	67.63 ± 0.276	0.423 ± 0.044	16.4 ± 0.178	61.63 ± 0.150	60.54 ± 0.193
80% v/v methanol	69.71 ± .885	0.521 ± 0.074	16.8 ± 0.272	63.31 ± 0.685	68.24 ± 0.483
70% v/v methanol	81.69 ± .924	0.607 ± 0.094	19.9 ± 0.393	70.69 ± 0.526	78.5 ± 0.475
100% ethanol	72.41 ± 0.675	0.558 ± 0.062	17.2 ± 0.213	64.73 ± 0.266	63.7 ± 0.483
80% ethanol	72.6 ± 0.543	0.549 ± 0.084	17.7 ± 0.174	65.12 ± 0.314	70.80 ± 0.553
70% ethanol	83.21 ± .885	0.780 ± 0.064	23.3 ± 0.172	73.71 ± 0.675	80.84 ± 0.453
Control(BHT)	74.31 ± 0.795	0.624 ± 0.061	18.2 ± 0.413	67.93 ± 0.186	65.7 ± 0.483

*=average of three estimations at each level

Table 5: Evaluation of *in vitro* antioxidant assay of different solvent extracts from air dried *Saussurea costus* roots

Solvent extract	*DPPH scavenging activity (%, mean ± SD)	*IC50 values (mg/mL, d.w.) of DPPH scavenging activity	*Ferric reducing power(µg/g, dw)	*Hydrogen peroxide scavenging activity (%, mean ± SD)	*IC50 values (µg/mL, d.w.) of H_2O_2 scavenging activity
100% methanol	61.67 ± 0.546	0.312 ± 0.032	13.8 ± 0.167	57.52 ± 0.143	57.43 ± 0.282
80% v/v methanol	62.41 ± .580	0.401 ± 0.064	14.6 ± 0.383	60.24 ± 0.584	64.13 ± 0.372
70% v/v methanol	75.73 ± .604	0.487 ± 0.083	16.8 ± 0.290	66.68 ± 0.415	74.55 ± 0.364
100% ethanol	66.54 ± 0.399	0.428 ± 0.051	14.5 ± 0.425	60.63 ± 0.386	60.45 ± 0.372
80% ethanol	67.6 ± 0.843	0.469 ± 0.074	15.5 ± 0.184	62.40 ± 0.203	67.60 ± 0.457
70% ethanol	79.51 ± .774	0.570 ± 0.063	19.6 ± 0.186	70.61 ± 0.564	76.64 ± 0.342
Control(BHT)	70.20 ± 0.684	0.425 ± 0.052	16.1 ± 0.523	65.82 ± 0.275	63.70 ± 0.372

*=average of three estimations at each level

The total phenolic content of air-dried, sun-dried and oven-dried *Saussurea costus* roots varied significantly ($P < 0.05$) with the oven-dried samples having the highest phenolic contents (Figure 1 and 2). This effect of drying method has been consistently demonstrated with respect to antioxidant yield, antioxidant activity and total phenolic content. The air-dried samples had been dried for 10 days, the sun-dried samples for 7 days while the oven-dried for 3 days indicating that as the length of drying time increased, antioxidant activity decreased. The use of higher temperatures for drying prior to extraction has been a focus of several studies [24, 25] reported that a drying temperature of 60°C (and lower) did not adversely affect total phenolics of mulberry leaf extracts, however, when temperatures of 70°C (and above) were employed total phenolics decreased significantly. Another study, this time involving tomatoes, thermal heating for 2, 15 and 30 minutes at 88°C had no significant effect on total phenolics [24]. While our study suggests shorter drying times (by employing heat, 40°C) is superior to an ambient- drying process, the point at which the temperature begins to significantly and adversely affect antioxidant activity is unknown and needs further investigation.

CONCLUSION

Based on the results obtained in the present study, it was concluded that aqueous ethanol and methanol from roots of *Saussurea costus* contain higher quantity of total phenolics and flavanoids.They give significant antioxidant activities to hydroxyl radical, superoxide radical, and DPPH radical. There was found a significant and linear relationship between the antioxidant activity and the content of flavonoids. Thus, the aqueous ethanol and methanol extract from roots of *Saussurea*

costus could be used as an antioxidant herb for adjuvant therapy. As the synthetic antioxidant BHT was forbidden being used in food due to its side effects on human, development of the natural antioxidants was meaningful and prospective. So they can be used as natural antioxidants after isolation and purification.

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