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ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF *Microtoena patchouli* LEAVES EXTRACT

L.D. Sharma[1] and M.R. Khan[2]

ABSTRACT

The present study was carried out in the year 2022 at Department of Botany, Pravabati College, Mayang Imphal, to analyse an antioxidant activity and total phenolic content of *Microtoena patchoulii* leaves extract and to determine the phyto-constituents using solvents such as methanol and ethanol. The total phenolic content in methanol and ethanol leaves extract in terms of gallic acid equivalent was 24.01 and 6.06 of extract powder respectively. Total antioxidant activity varied among the solvent used in extract with methanol which have higher activity than ethanol extract. The total antioxidant activity of methanolic and ethanolic extracts of leaves was found highest (48.3 ± 1.01 µg AAE mg^{-1} and 24.6 ± 0.62 µg AAE mg^{-1} respectively) in highest concentration of 90 µg ml^{-1} of extract. The present study indicates the leaves of *Microtoena patchouli* possessed antioxidant activity. Total phenolic content was highest in methanol extract than ethanol extract. Total antioxidant activity was highest in methanolic leaves extract and ethanolic leaves extract have more reducing power than methanolic leaves extract.

(Key words: Antioxidant, phenolic, methanol, ethanol, *Microtoena patchouli*)

INTRODUCTION

In spite of much progress made in synthetic drug research, plants and their products are still considered to be the major sources of medicaments and have extensive use in the pharma industry (Harvey, 2008; Meena *et al*., 2009). Most modern medicines are derived from plants and their products obtained by applying modern technologies to traditional practices (Sucher and Carles, 2008). The use of plants is customary in Indian systems of medicine like Ayurveda, Unani, Sidda and many other indigenous and folk practices (Kumara *et al*., 2012).

Microtoena patchouli generally called patchouli is the native range in Himalaya to China and Indo – China. It is herb 1 – 2 m tall, with spreading hairs, velvety, base somewhat woody, much branched. It is an aromatic herb need for cough, asthma, enteritis. More than 9000 native plants have been identified and recorded for their curative properties and about 1500 species are known for their aroma and flavour. Essential oil based products or natural aroma chemicals are in higher demand in the cosmetic, food, perfumes and pharmaceutical industries, and more than 250 types of essentials, at a value of 1.2 billion USD, are traded annually on the international market (Swamy and Sinniah, 2016). Essential oils have tremendous business potential on the global market owing to their unique flavour and fragrance properties and also biological activities (Swamy *et al*., 2015). *M. patchoulii*, oil in the plant is widely used in Traditional Chinese Medicine (TCM) as it offers various types of pharmacological activities (Donelian *et al*., 2009). It has also been reported to strong than the immune activity and resistance to bacterial action (Hu *et al*., 2006). The dry leaves of this plant on steam, distillation, yield an essential oil and important ingredient in many fine fragrance products such as perfumes as well as in soaps and cosmetic products (Singh *et al*., 2002). Results of high performance thin layer chromatography (HPLC) studies indicated that the ethyl acetate extract of *Pogostemon parviflorus* leaves included triterpenes, as 10 and 15 peaks of ultra violet (uv) absorption were observed in 254 nm and 366 nm, respectively. Hence, triterpenes may be responsible for anti –dermatophytic activity (Sadeghi – Nijad *et al*., 2010). In addition, Patchouli oil is widely used in the manufacturing of soaps, scents, body lotions and detergents (Kumaraswamy and Anuradha, 2010). Patchouli also possesses insecticidal, antibacterial and antifungal properties (Chakrapani *et al*., 2013; Kalra *et al*., 2006; Kumara and Anuradha, 2011). Some of its other biological activities include antimicrobial, antioxidant, analgesic, anti – inflammatory, anti platelet, antithrombotic, aphrodosiac, antidepressant, antimutagenic, antiemetic, fibrinolytic and cytotoxic activities (Lui *et al.,* 2009; Priya *et al*., 2014). Seeing its biological activities and the major sources of medicaments and have extensive in the pharma industry, the present study was carried out to analyze the phytochemical constituent, total phenolic content and antioxidant activity of *Microtoena patchoulii* collected from *Laphupat tera*, Imphal west district of Manipur.

1. Asstt. Professor, Dept. of Botany, Pravabati College, Mayang Imphal, Manipur, India.
2. Assoc. Professor, Dept. of Botany, Lilong Haoreibi College, Lilong, Manipur, India (Corresponding author)

MATERIALS AND METHODS

Collection of plant

Leaves of *Microtoena patchoulii* were collected from the *Laphupattera* Imphal west district, Manipur. Authentication of the plant sample was done in Manipur University, Department of Life Sciences (Botany). The leaves were washed thoroughly with tap water followed by distilled water. Then the rhizomes were dried under shaded at room temperature and ground into powder.

Preparation of solvent extracts for qualitative screening

40 g of ground samples were weighed and carried out the process by using 400 ml of methanol and ethanol in soxhlet apparatus respectively. Soxhlet extraction has been used widely (Redfern *et al*., 2014). The extracts were concentrated by evaporation and stored prior to phytochemical screening.

Phytochemical screening

The extracts were subjected to phytochemical screening to test presence of phytoconstituents such as amino-acids, carbohydrates, proteins, phenol, flavonoids, tannins, steroids and terpenoids, saponins, oils and fats, phlobatanins, etc.

The phytochemical tests were carried out using the standard procedures (Khan *et al.,* 2023; Sharma *et al*., 2023; Bhaigyabati *et al*., 2017).

Determination of total phenolic content

The Folin-Ciocalteu reagent method was used to determine the amount of phenol content in methanol and ethanol leaves extract of *Microtoena patchoulii* (Bag *et al*., 2016). 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (2% w/v) were added to 0.5 ml of the sample (3 replicates) of leave extract solution (1mg ml^{-1}). The resulting mixture was incubated at 45°C for 15 min. The absorbance of sample was measured at 760 nm using UV Visible Spectrophotometer (UV-2700). Gallic acid (50-300 ì g ml^{-1}) was used as a standard compound. The gallic acid standard calibration curve was established by plotting concentration (ìg ml^{-1}) versus absorbance (nm) (y= 0.009675X + 0.004840; R2= 0.9584), where y is absorbance at 760 nm and x is concentration (Figure 1). Total phenolic content in the plant extract was expressed as gallic acid equivalent (mg of gallic acid equivalent g^{-1} of sample) and was calculated by the formula:

$$T = (C \times V)/M$$

Where, T = total content of phenolic compounds, mg g^{-1} plant extract, in GAE; C = concentration of gallic acid established from the calibration curve, µg ml^{-1}; V = volume of extract, ml; M = weight of the plant (Bhaigyabati *et al*., 2017).

Estimation of reducing power

Different concentrations of the extracts were prepared and mixed with 2.5 ml phosphate buffer and potassium ferricyanide and the mixture was kept at 50°C in water bath for 20 min. After cooling 2.5 ml of 10% trichloroacetic-acid was added and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and freshly prepared 1% ferric chloride solution (0.5 ml). The absorbance was measured by using UV spectrophotometer at 700 nm. Control was prepared in similar manner excluding samples. Ascorbic acid (0.5 mg ml^{-1}) at various concentrations was used as standard (Khan *et al*., 2023, Sharma *et al*., 2023).

Determination of total antioxidant activity

The total antioxidant activity of the extracts was evaluated by using the phosphomolybdenum method (Khan *et al*., 2023; Sharma *et al*., 2023). 0.3 ml of the methanolic and ethanolic extract sample (1mg ml^{-1}) as well as ascorbic acid (0.5 mg ml^{-1}) was mixed with 3.0 ml of the reagent solution (0.6 M sulphuric acid, 28 nM sodium phosphate and 4 nM ammonium molybdate) separately and the mixture was incubated at 95°C for 90 min under water bath. Absorbance of all the mixtures was measured at 695 nm after cooling. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid in ìg$ml^{-1}$of extract. Total antioxidant activity was calculated by using the formula.

Total antioxidant = O.D. of test x concentration of standard in µg X made up volume of sample (Khan *et al*., 2023; Sharma *et al*., 2023).

Statistical analysis

All sample determinations were conducted in triplicates and the results were calculated as mean ± standard deviation (SD) (Sharma *et al*., 2023).

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening of methanol and ethanol of *M. patchouli* leaves revealed the presence of phytoconstituents as listed in Table 1.

Phytochemicals *viz*., amino acids, carbohydrates, steroids and terpenoids, saponins, phenolic compounds and tannins were present in methanol except alkaloids, proteins, flavonoids, cardiac glycosides, oils and phlobatanins and ethanol *viz*., amino acids, carbohydrates, saponins, phenolic compounds and tannins were present except alkaloids, proteins, flavonoids, steroid and terpenoids, cardiac glycosides, oils and phlobatanins extracts of *Microtoena patchoulii* leaves. Important phytochemical considered as active medicinal phytochemical were present in the sample that showed a high level of its possible medicinal value (Khan *et al*., 2023, Sharma *et al*., 2023).

Total phenolic content

The phenolic compounds act as free radical terminators and the mechanism of action are through scavenging or chelating process (Platzer *et al*., 2021, Khan *et al*., 2023). Phenolic compounds are having wide bioactivity including antioxidant properties/activity. The antioxidant

activity of phenolic compound is due to hydroxyl functional group, however other factors eg., presence of electron withdrawing or releasing group in the aromatic ring having hydroxyl moiety will increase or decrease the activity. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties (Bag *et al*., 2016). The present study reveals that the total phenolic content in methanol and ethanol leaves extract in terms of gallic acid equivalent was 24.01 and 6.06 of extract powder respectively. Result indicates that methanol extract of leave of *Microtoena patchouli* showed higher total phenolic content than ethanol (Table 2). The total phenolic content varied according to the solvent use in extract.

Determination of reducing power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Figure 2 shows the dose dependent reducing power activity of *Microtoena patchoulii* methanol and ethanol leaves extract at different concentrations and using the potassium ferricyanide reduction method. The concentration of extract ranged from 10-90 µg level. As the concentration of extract increased the reducing power, also increased in both the solvent and attained maximum at 100 µg concentration. Increasing absorbance indicates an increase in reductive ability. Ethanolic extracts showed higher reducing power than methanolic extracts. The reducing power activity is due to the presence of reductones (phenolics). As reducing power assay measures the electron donating capacity of an antioxidant, it is associated with the presence of reductones. Reductones exhibit antioxidant action by breaking the chain reactions by donating a hydrogen atom and also reported to react with certain precursor of peroxide thereby preventing peroxide formation (Olayinka *et al*., 2010, Khan *et al*., 2023).

Total antioxidant activity

Table 3 shows that total antioxidant activity varied among the solvent used in extract with methanol extract having higher activity than ethanol extract and also indicate the total antioxidant activity increased with the increase in concentration of extract. The total antioxidant activity of methanolic and ethanolic extracts of leaves was found highest (48.3 ± 1.01µg AAE $mg^{-1}$and 24.6 ± 0.62 µg AAE mg^{-1} respectively) in highest concentration of 90 µg ml^{-1} of extract and lowest (27.9 ± 0.75 µg AAE mg^{-1} and 19.5 ± 0.49 µg AAE mg^{-1} respectively) in lowest concentration of 10 µg ml^{-1} of extract. However, the total antioxidant activity depends on the solvent type and its concentration used in extraction. The antioxidant quality or activity of plant-derived products has greatly influenced by the extraction factors like the method of extraction, temperature and solvent used. Several studies evidently show that the effectiveness of antioxidant activity of most plant products was significantly increased by using the aqueous mixtures of organic solvents like ethanol, methanol, acetone, isopropanol, or acetonitrile with water rather than using water alone for extraction (Thamizhinyan *et al*., 2019).

Table 1. Phytochemical constituents of *Microtoena patchoulii* leaves

Phytochemicals	Test performed	Solvent	
		Methanol	Ethanol
Amino acids	Ninhydrin	+	+
Alkaloids	Mayre's test	-	-
Carbohydrate	Benedict's test	-	-
	Fehling's test	+	+
Proteins	Xanthoproteic test	-	-
	Biuret test	-	-
Flavonoids	Alkaline reagent test	-	-
	Lead acetate test	-	-
Steroids and terpenoids	Salkowski's test	+	-
Saponins	Froth test	+	+
Cardiac glycosides	Keller Killiani test	-	-
Phenolic compounds	Lead acetate test	+	+
	Ferric chloride test	+	+
Tannins	Lead acetate test	+	+
	Ferric chloride test	+	+
Oils	Translucent test	-	-
Phlobatanins	HCl test	-	-

Key + =presence, - = absence

Table 2. Total phenolic content in *Microtoena patchoulii* leaves extract

Solvent	Total phenolic content (mg GAE g^{-1} of extract)
Methanol	24.01±0.087
Ethanol	6.06±0.56

Assays were performed in triplicate. Values are expressed as means ± SD

Table 3. Total antioxidant of *Microtoena patchoulii*

Concentration ($\mu g\ ml^{-1}$)	Total antioxidant activity in μg AAE mg^{-1} of extract	
	Methanol	Ethanol
10	27.9 ± 0.75	19.5 ± 0.49
30	36.0 ± 0.75	20.4 ± 0.43
50	38.7 ± 0.79	21.9 ± 0.44
70	42.6 ± 1.07	23.4 ± 0.59
90	48.3 ± 1.01	24.6 ± 0.62

Assays were performed in triplicate. Values are expressed as means ± SD

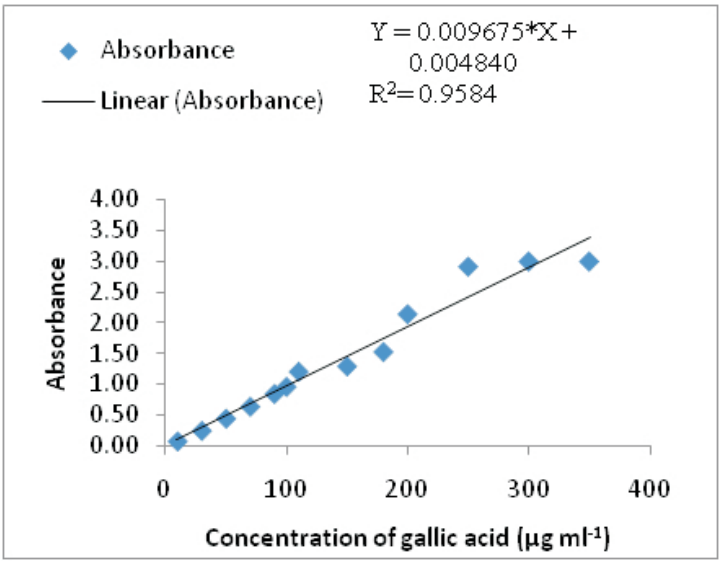

Figure 1. Standard curve of gallic acid ($\mu g\ ml^{-1}$)

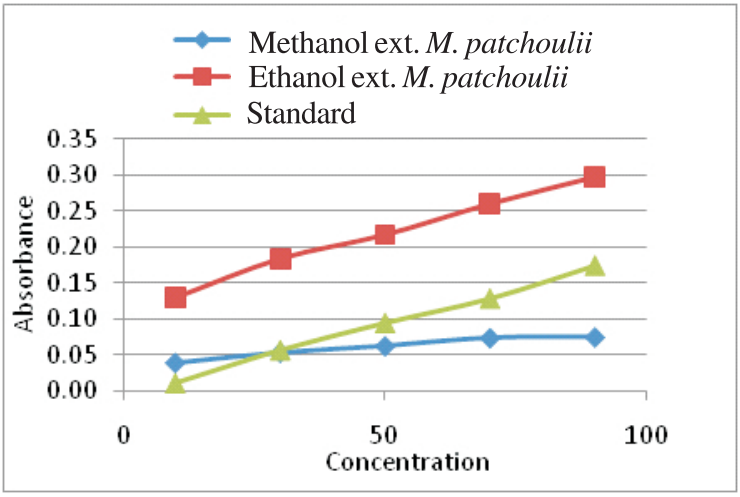

Figure 2. Reducing power of methanolic and ethanolic extracts of *Microtoena patchoulii*

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