Evaluation of Phytochemical contents and antioxidant activity of *Mentha spicata* (spearmint) Grown in Kano, Nigeria

ABSTRACT

Aim: *Mentha spicata* (spearmint) is a plant that spreads mainly in the temperate and sub-temperate zones of the world including Africa and is used in pharmaceutical and food industries. It has numerous medicinal importance uch as antibiotic, anti-inflammatory, anti-diabetic, anti-hyperlipidaemic and many more. This research examines the phytochemical and antioxidant property of *Mentha spicata*. Methodology: Sample of *Mentha spicata* leaves were collected fresh from the cultivated farm and was extracted using distilled water prior to analysis. The phytochemicals determination was carried out in accordance with methods described by Velavan while the antioxidant property was evaluated using Diphenylpicrylhydrazyl (DPPH) radical scavenging assay. Results: The result showed that the aqueous extract contain alkaloids, phenols, flavonoids, tannins, saponins glycosides, Terpenoids and anthraquinones at varying concentrations. The antioxidant activity was measured in percentage at different concentration of the extract as follows; 25mg/dl (20.94±0.05%), 50mg/dl (10.78±0.02%), 75mg/dl (50.74±0.04%) and 100mg/dl (17.83±0.06%) respectively. The highest antioxidant activity was observed in 75mg/dl concentration. Conclusion: The antioxidant activity observed could be attributed to the presence of flavonoids and phenols. Therefore, Mentha spicata can be used as a source of antioxidant and could improve the health status of individuals.

Key words: *Mentha spicata*, Evaluation, phytochemicals, antioxidant,

1. Introduction

The use of traditional herbs is gaining more attention owing to general believe that the traditional herbs posses medicinal attributes among which are antioxidant, anti-diabetic, anticancer, antimicrobial and hypolidemic. Extracts of traditional herbs are often providing opportunities for drug discovery [1]. Therefore, various parts of different types of plants are been used in the treatment of diseases [2]. Spearmint (Mentha spicata) is a member of Lamiaceae that spreads mainly in the temperate and sub-temperate zones of the world including Asia, Africa, Australia, North America, and Europe [3]. It is regarded as an important source of essential oils that is widely used as a raw material in pharmaceutical industries as well as [3]. The genus *Mentha* includes 25 to 30 species such as spearmint, peppermint, wild mint, corn mint, curled mint, bergamot, American mint, Korean mint, etc. of which spearmint is the most common of them [4, 5]. Various parts of Mentha species are used in folk medicine over a long period of time in the treatment of many dysfunctions such as those of gastrointestinal tract [6]. Mentha spicata (spearmint) has been reported to possess many medicinal properties such as antibacterial and antioxidant [3, 7, 8], stomach pain-relieving agent, antispasmodic, digestive and carminative [9], hypolipidemic and anti-diabetic [10].

Research has shown that the mint main chemical compounds includes limonene, cineole, menthone, menthofuran, isomenthone, menthylacetate, isopulegol, menthol, pulegone and carvone [11]. Areias [12] has however reported mint to be rich in other constituents such as flavonoid glycoside (eg. Narirutin, Luteolin-7-o-rutinoside, Isorhoifolin and Hesperidin), polyphenols (e.g Rosmaric acid, Eriocitrin, Cinamic acid, Caffeic acid and Narigenin-7-oglucoside); luteolin-diglucoronide and eriodictyol glucopyranosyl-rhamnopyranoside. The amount of mint compounds is different in various species [13]. Various factors including physiological variations, environmental conditions, geographic differences and genetic factors cause differences in chemical composition of these plants [13]. This study is therefore aimed at determining the phytochemical and antioxidant property of Mentha spicata (Spearmint).

2. MATERIALS AND METHODS

2.1 Sample collection and

Fresh leaves of spearmint were collected from the cultivated farms of Aminu Kano College of Islamic and Legal Studies and the T-junction of Kofar Famfo within Kano metropolis. The samples were identified and authenticated prior to analysis and then freeze dried. The dried leaves were ground in to fine powder using mortar and pestle and stored in polyethene bag at room temperature prior to analysis.

2.2 Extraction of Spearmint Extract

Extraction of the powder Spearmint was conducted using distilled water. The extracts were concentrated in vacuum using auto rotary evaporator 50L RE50V2. After concentration, the extract was allowed to dry and kep in refrigerator for further analysis as described by [14, 15].

2.3 Preliminary Phytochemical Screening

Qualitative phytochemical tests were carried out by using standard procedure of preliminary phytochemical screening in accordance with the methods described by [14, 15]. The screening was carried out for alkaloids, polyphenols, flavonoids, tannins, saponins, glycosides, terpenoids, anthraquinones and anthocyanins.

2.4 Qualitative Analysis on Phytochemical Constituent of Mint Leaves

5g of the *Mentha spicata* extract was measured using digital weighing balance and dissolved in 50ml of distilled water. The mixture was shaken gently using mechanical shakerand was allowed to dissolve for about five (5) minutes and then filtered using Whatman No. 5 filter paper. The filtrate was then subjected to the following qualitative tests.

2.4.1 Test for Alkaloids

Few drops of 1% HCl were added to the filtrate to which 5 drops of freshly prepared Dragen dorrf‟s reagent (potassium bismuth iodide solution) was added. The solution (1 cm^3) was treated with Wagners reagent (solution of iodine in potassium iodide). Formation of a precipitate indicated the presence of alkaloids [15].

2.4.2 Test for Anthraquinones

5ml of the filtrate was hydrolyzed with diluted Conc. H_2SO_4. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones [15].

2.4.3 Test for Phenols

Addition of 150 µl of 5% lead acetate to the filtrate leads to the formation of yellow precipitates indicating the presence of phenolic compounds [15].

2.4.4 Test for Tannins

0.5g of the dried powdered of Mentha spicata extract was boiled in 20 cm^3 of water in a test tube and then filtered. 150 µl of 0.1% ferric chloride were added. Formation of brownish green coloration indicated the presence of tannins [15].

2.4.5 Test for Saponins

2 g of the powdered Mentha spicata extract was boiled in 20 ml of distilled water in a water bath and then filtered. 10 cm^3 of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drop s of olive oil and shaken vigorously; formation of emulsion indicates the presence of saponin [15].

2.4.6 Test for Flavonoids

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of the powdered Mentha spicata extract followed by addition of concentrated H_2SO_4. A yellow coloration observed within 10 minutes in each filtrate indicated the presence of flavonoids. The yellow colouration disappeared on standing [15].

2.4.7 Test for Terpenoids (Salkowski Test)

5ml of the filtrate of Mentha spicata extract was mixed in 2 cm^3 of chloroform; 3 cm^3 of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

2.4.8 Test for Cardiac Glycosides (Keller-Killani test)

5ml of the filtrate of the filtrate of Mentha spicata extract was treated with 2 cm^3 of glacial acetic acid containing one drop of ferric chloride solution. This was then treated with 1 cm^3 of concentrated sulphuric acid. A brown ring of the interface indicated a deoxysugar characteristic of cardenolides [15].

2.5 Quantitative Analysis of Phytochemical Constituent

Six of the phytochemicals namely; alkaloids, phenols, flavonoids, tannins, saponnins and cardiac glycosides were subjected quantitative analysis using standard methods.

2.5.1 Determination of Alkaloids

5 g of the extract of *Mentha spicata* was weighed into a 250 cm^3 beaker and 200 cm^3 of 10% acetic acid and ethanol was added and covered and was allowed to stand for 4 hr. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed [15].

2.5.2 Determination of Total Phenols by spectrophotometric method

The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15 minutes. 5 cm^3 of the extract was pipetted into a 50 cm^3 flask, 10 $cm^3$of distilled water was added. This was followed by the addition of 2 $cm^3$of ammonium hydroxide solution and 5 cm^3 of concentrated amyl alcohol.. The samples were made up to mark and left to react for 30 minutes for color development. This was measured at 505nm using spectrophotometer [15].

2.5.3 Determination of Tannins

500 mg of the Mentha spicata extract was weighed into a 50 cm^3 plastic bottle followed by the addition of 50 cm^3 of distilled water and the mixture was shaken for 1hr in a mechanical shaker. This was filtered into a 50 cm^3 volumetric flask and made up to the mark. Then 5 cm^3 of the filtrate was pipetted out into a test tube and mixed with 2 cm^3 of 0.1 M $FeCl_3$ in 0.1N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 720 nm within 10min [15].

2.5.4 Determination of Saponin

20 g of Mentha spicata extract was placed into a conical flask and 100 cm3 of 20% aqueous ethanol was added. The mixture was heated over a hot water bath for 4hr with continuous stirring at about 55^0C. The mixture was filtered and the residue re-extracted with another 200 cm^3 of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^0C. The concentrate was transferred into a 250 cm^3 separating funnel and 20 cm^3 of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 cm^3 of n-butanol was added. The combined n-butanol extracts were washed twice with 10 cm^3 of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the solution was dried in the oven to a constant weight [15].

2.5.5 Test for Flavonoids

10 g of Mentha spicata extract was extracted repeatedly with 100 cm^3 of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight [15].

2.5.6 Determination of Cardiac Glycosides

A tincture of the sample was prepared by preparing 10% extract in 70% alcohol by shaking 1g of pulverized Mentha with 10 cm^3 70% alcohol. The mixture was left overnight with occasional shaking for 2hr and then filtered. 10 cm^3 of the purified filtrate transferred in to a dry stopped Erlynmeyer flask was added to 10 cm^3 of Baltet's reagent. The blank was prepared at the same time using 10 $cm^3$of distilled water instead of the purified filtrate and 10 cm^3 of Baltet's reagent. This was made to stand for 1hr, for maximum color development. The solution was diluted with 20 cm^3 of distilled water and mixed. The intensity of the color obtained was measured at 495nm using spectrophotometer. The color was stable for several hours. The difference between experiment and blank (E-B) is equal to the original reading. The percentage total glycoside was calculated using the absorptivity of digitoxin = 170, similarly treated at 495 nm as follows:

% Total cardiac glycoside = (A x 100 / 17) g% Calculated as digitoxin.

Where A = absorbance of the colour at 495nm.

2.6 Determination of Antioxidant Activity

For antioxidant activity determination, 20g of Mentha spicata extract was dissolved in 100 cm^3 absolute methanol and soaked overnight. The resulting solution was filtered and transferred to clean vessel and subsequently evaporated to dryness in order to obtain the extract [16].

2.6.1 DPPH Radical Scavenging Activity

Diphenylpicrylhydrazyl (DPPH) radical scavenging activity was measured as described by [17] with some modifications. The reaction mixture up to 3 cm^3 containing 0.2 cm^3 of DPPH and 2.8 cm^3 of test solution at various concentrations i.e. (20, 40, 60, 80,100mg/ml) of the extract fractions was incubated at 37°C for 30minutes. The absorbance of the resulting solution was measured at 517nm using spectrophotometer (Beckman model DU-40) The percentage inhibition of DPPH radical scavenging activity was calculated by comparing the results of the test with those of the control (not treated with extract) using the following equation: DPPH scavenging activity = (Ac−As)/ Ac × 100 Where Ac= Absorbance of control, As= Absorbance of sample [18].

2.7 Statistical Analysis

Results were analyzed statistically using Analysis of variance (ANOVA). Difference of mean values were determined at $P < 0.05$ level of significance.

3. RESULTS

3.1 Phytochemical Contents

The results for qualitative and quantitative phytochemical constituents of aqueous extract of Mentha spicata are presented in tables 1 and 2.

Table 1: Qualitative Phytochemical Constituents of Mentha spicata grown in Kano, Nigeria

Phytochemicals	Status
Alkaloids	+
Polyphenols	+
Flavonoids	+
Tannins	+
Saponins	+
Glycosides	+
Terpenoids	+
Anthraquinones	+

Table 2: Quantitative Phytochemical Constituents of Mentha spicata grown in Kano, Nigeria

Phytochemicals	**Concentration (mg/g)**
Flavonoids	8.29 ± 0.18
Alkaloids	32.09 ± 0.08
Phenols	7.96 ± 0.04
Tannins	2.15 ± 0.15
Saponin	1.51 ± 0.47
Cardiac glycosides	2.54 ± 0.04

Results are in mean ± standard deviation (n=3).

3.2 Antioxidant Activity

The antioxidant activity of Mentha spicata extract used in the study was measured using Diphenylpicrylhydrazyl radical (DPPH) scavenging assay. This was measured at various concentrations in mg/dl (i.e 25, 50, 75 and 100). The highest antioxidant activity was observed at 75 mg/dl concentration (table 3). The antioxidant activity is based on the reduction of DPPH free radical by an antioxidant [16].

Table 3: Antioxidant Activity of Mentha spicata Extract Grown in Kano, Nigeria

Concentration in mg/dl	% Inhibition
25	20.94±0.05
50	10.78±0.02
75	50.74±0.04
100	17.83±0.06

Results are in mean ± standard deviation (n=3).

4. DISCUSSION

As shown in the table 1 above, the aqueous extract of Mentha *spicata* showed the presence of eight phytochemicals, namely alkaloids, phenols, flavonoids, tannins, cardiac glycosides, saponins, terpenoids and anthraquinones. Six of the eight phytochemicals determined were subjected to quantitative analysis and the

results indicated that the extract of Mentha spicata contain varying amount of phytoconstituents such as flavonoids (8.29 ± 0.18 mg/g), alkaloids (32.09 ± 0.08 mg/g), phenols (7.96 ± 0.04 mg/g), tannins (2.15 ± 0.15 mg/g), saponins (1.51 ± 0.47 mg/g) and glycosides (2.54 ± 0.04 mg/g) (table 2). This phytochemicals have various medicinal properties. *Mentha spicata* possesses astringent, antiseptic, antispasmodic, anti-inflammatory, analgesic, and anti-carcinogenic properties [19]. The anti-inflammatory effect could be attributed to the presence of alkaloids, flavonoids, phenolic acids, and tannins [20, 21, 22, 23]. The anti-carcinogenic activity could be attributed to the presence of Terpenes and saponin [24, 25]. The analgesic properties can be attributed to the presence of some alkaloids such as morphine [26]. The antispasmodic effect may result from the presence of phenolic acids [20]. The astringent property of Mentha spicata is perhaps due to the presence of tannins [27], which may also be responsible for the antiseptic effect [22]. The present study is in agreement with various studies that plants contain a vast number of phytochemicals [28, 29].
The concentration of flavonoids (32.09 ± 0.08 mg/g) and phenols (7.96 ± 0.04 mg/g) determined in the current study are higher than those reported by [30]. Plant polyphenols such as flavonoids have vast array of biological activities, including anti-inflammatory, anti-oxidative and free radical-scavenging property [31]. Dietary flavonoids possess anticancer, anti-inflammatory and anti-infectious properties and can be used to prevents obesity and as potential anti Covid-19 [32].

The antioxidant activity of the Mentha spicata extract used in this study has indicated that, the extract possess an antioxidant property. However, the result showed that Mentha spicata exhibits the highest antioxidant activity at 75 mg/dl concentration. Antioxidants are indispensable for cellular response in order to deal with oxidative stress under physiological conditions and can be effective through several ways [33]. They act as preventive (preventing the formation of lipid free radicals), chain breaking antioxidants (interfering with the circulation of the autoxidation chain reaction), as singlet oxygen quenchers, through synergism with other antioxidants; as reducing agents which convert hydroperoxides into stable compounds; as metal chelators that convert metal pro-oxidants (iron and copper derivatives) into stable products; and finally as inhibitors of pro-oxidative enzymes (lipoxygenases) [34].

The higher antioxidant activity (50.74 mg/dl) observed in this study could be attributed to the presence of flavonoids and phenolic compounds in Mentha spicata extract. Flavonoids have multi beneficial activities including an antioxidant activity [35]. The antioxidant activities of flavonoids and phenolic acids and further suggested that extracts of plants containing flavonoids and phenolic acids can be considered as promising antiaging sources for use in cosmetic formulations [35].

5. Conclusion

Extract of Mentha spicata is rich in phytochemicals that have numerous medicinal importance that could promote health and can be used as a source of antioxidants. The medicinal properties of Mentha spicata could therefore be attributed to the presence of phytochemicals determined such as alkaloids, phenols, flavonoids, tannins, saponins, Terpenoids and anthraquinones.

Competing interests disclaimer:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

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