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A STUDY ON PHYTOCHEMISTRY OF *MORINGA OLEIFERA* DRUMSTICKS: QUALITATIVE AND QUANTITATIVE EVALUATION

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Abstract

Medicinal plants are an excellent source as they provide a wide variety of possible therapeutic compounds that are both diversified and reasonably safe, compared to manufactured pharmaceuticals. In the current study we aimed to evaluate the qualitative and quantitative phytochemical analysis of drumstick parts of *Moringa oleifera* plant. The aqueous (*aq.*) extract of drumstick parts of Moringa oleifera plant was prepared by maceration of 100 g of sample powder of drumstick parts of *M. oleifera* in two liters of distilled water was macerated for 24 hours followed by filtration using Whatman filter paper No.1. The qualitative and quantitative phytochemical analysis was carried for *aq.* drumstick extract of *M. oleifera.* Qualitative phytochemical analysis of *aq.* drumstick extract of *M. oleifera* revealed alkaloids, flavonoids, sterols, and tannins as major phytochemicals found in *aq.* drumstick extract of *M. oleifera.* Quantitative phytochemicals estimation of *aq.* drumstick extract of *M. oleifera* revealed that tannins were found to be present in highest quantities i.e., 24.89 ± 2.41% followed by flavonoids, sterols, and alkaloids in 6.15 ± 0.28%, 5.04 ± 0.15%, and 3.65 ± 0.16% respectively. In conclusion, study findings clearly demonstrated that *aq.* drumstick extract of *M. oleifera* contains secondary metabolites *viz.* alkaloids, flavonoids, sterols, and tannins that are beneficial in treatment of various human ailments. Hence, Moringa

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drumsticks could be explored in the traditional medicine as antipyretic, antioxidant, analgesic agents, immune enhancers, and hormone modulators.

Keywords: *Moring oleifera*, Drumsticks, *Aq.* extract, Phytochemicals, Traditional medicines

Introduction

Herbal preparations have been used by humans since time immemorial for treating various medical disorders. More than half a million unique species of plants on the earth present a rich resource of phytochemicals with potential therapeutic properties. Secondary metabolites are produced by plants as a defense against injurious pathogenic and environmental conditions. The major advantage of synthetic chemicals is that they are relatively much safer, easily accessible, and cost less. Initially exploited for antimicrobial use, the applications have expanded to a wide range of conditions including cancer, inflammatory disorders, immune disorders, and diabetes.[1-3]

Moringa oleifera is a common plant species of mainly tropical climates. It is a small graceful, deciduous plant with thin foliage and can grow up to 8 m height (Figure 1A and 1B). Almost every part of the plant has been employed for nutritional and medicinal uses. Traditional medicine and now increasingly, modern science have recognized the unique composition of this plant and its efficacy in the treatment of various diseases. In particular, the extracts of the plant are known to possess antiproliferative, anti-inflammatory, antimicrobial, antioxidant, and osteoprotective effects.[4-7]

Figure 1A: Showing *Moringa oleifera* plant

Figure 1B: Showing *Moringa oleifera* drumsticks

Moringa oleifera (Lam.) has many common names such as ben oil, drumstick, horseradish, and miracle tree. *M. oleifera* is a widely distributed species of the family Moringaceae. Moringa is native to Western and sub-Himalayan regions, India, Pakistan, Asia and Africa and it is distributed throughout the world in arid and semi-arid climate.[8] Moringa trees are having a remarkable range of medicinal properties as high as nutritional values. Most parts of the plant *viz.* leaves, seeds, fruit or pods, roots, stem, and bark are used as medicines or foods in various countries with especial references to the traditional communities. The leaves are rich source of both macro- and micronutrients, such as protein and many vitamins.[9] Fresh leaf juice inhibits the growth of human pathogens.[10] The seeds also show antimicrobial

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activity.[11] Fruits or pods have wide spectrum of antimicrobial and antifungal activities against common pathogens.[12] The roots have been reported to have antispasmodic and antimicrobial activity and used for diarrhea treatment.[13]

Moringa contains specific plant pigments., alpha-carotene and beta-carotene, lutein and phytochemical constituents such as alkaloids, flavonoids, saponins, sterols, phenols and tannins. The therapeutic effects of *M. oleifera* could be due to the combined actions of various bioactive components found in the plant, including trace metal ions, vitamins, alkaloids, polyphenols and other elements,[14] and they collectively act on broad physiological processes including metabolism and immunity.[15] With this background, the present study was conducted to evaluate the qualitative and quantitative phytochemical analysis of drumstick parts of *M. oleifera* plant.

Materials and Methods

Collection of Plant Material

The drumsticks of *M. oleifera* were purchased from the local market of Bengaluru, Karnataka, India and washed several times with running tap water to remove adhered dirt and debris, and then shade dried at room temperature. Seeds were removed from the dried drumsticks of *M. oleifera*, and then crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

100 g of sample powder of drumsticks of *M. oleifera* was macerated in 2 liters of distilled water for 24 hours. The solution was decanted and filtered using Whatman filter paper No.1. The filtrate was dried in oven at 40°C for 4 days. The dry extract of drumsticks of *M. oleifera* was preserved in airtight containers and stored at room temperature until further use. The dry extract of drumsticks of *M. oleifera* was again was reconstituted in distilled water and used for the qualitative analysis.[16]

Qualitative Analysis of Phytochemicals

Test for Alkaloids

10 mL of *aq.* drumsticks extract of *M. oleifera* was evaporated to dryness. 2 mL of 2% HCl was added to the dried residue. Then few drops of Wagner's reagent was added. Reddish brown precipitation showed the presence of alkaloids in *aq.* drumsticks extract of *M. oleifera.*[17]

Test for Flavonoids

2 mL of *aq.* drumsticks extract of *M. oleifera* was dissolved in diluted 10%NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.[18] Few drops of (1%) lead acetate solution was added to 1 mL of the *aq.* drumsticks extract of *M. oleifera*. Intense white precipitation confirmed the presence of saponins.

Test for Saponins

Few drops of (1%) lead acetate solution was added to 1 mL of the *aq.* drumsticks extract of *M. oleifera*. Intense white precipitation confirmed the presence of saponins.[19]

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20 mL of distilled water was added to 1 mL of *aq.* drumsticks extract of *M. oleifera* in graduated cylinder, and resulting solution was shaken well for 5 to10 minutes. Formation of stable foam confirmed the presence of saponins.

Test for Sterols

2 mL of Conc. H_2SO_4 was added to 2 mL of *aq.* drumstick extract of *M. oleifera*. Formation of red precipitate is positive for the presence of sterols.[20]

Test for Tannins

1 mL of 3% of $FeCl_3$ was added to 1 mL of the *aq.* drumstick extract of *M. oleifera.* Development of brownish green color is positive test for tannins.[21]

Quantitative Estimation of Phytochemicals

Alkaloids

5 g of *aq.* drumstick extract of *M. oleifera* was weighed into beaker and 200 mL of 10% acetic acid in ethanol was added. The mixture was covered and allowed to stand for 4 hr. After that the mixture was filtered and the filtrate was concentrated on water bath at 100ºC to one-quarter of the original volume. Conc. NH_4OH was added to the extract in the form of drops until precipitation is completed. After settlement, the extract was filtered and the precipitate washed with dilute NH_4OH and then dried and weighed. The quantity of alkaloids in *aq.* drumstick extract of *M. oleifera* was expressed as percentage.[22] Estimation was conducted in three replicates and mean values are represented.

Flavonoids

100 mL of 80% *aq.* methanol was used to extract flavonoids from 10 g of *aq.* drumstick extract of *M. oleifera* sample. The mixture was filtered by using Whatman filter paper No. 42. The filtrate was evaporated until dried, and then weighed until constant weight was achieved.[23] The quantity of flavonoids in *aq.* drumstick extract of *M. oleifera* was expressed as percentage. Estimation was conducted in three replicates and mean values are represented.

Saponins

20g of *aq.* drumstick extract of *M. oleifera* sample was put into a 250 mL conical flask and 100 mL of 20% ethanol was added. The solution was heated over water bath at 100ºC for 4 hours with continuous stirring at 55°C. The solution was then filtered and the residue re-extracted with another 200 mL of 20% ethanol. The combined extract was reduced to 40 mL over a water bath. The concentrate was transferred into a 250 mL separating funnel and 20 mL of diethyl ether was added to the extract and was vigorously shaken. The aqueous layer was recovered while the ether layer was discarded and the purification process was repeated. 60 mL of n-butanol was added, the combined extract was washed twice with 10 mL of 5% NaCl. The remaining solution was heated in a water bath, and after evaporation, the sample was dried in the oven at 105ºC to a constant weight.[24] The quantity of saponins in *aq.* drumstick extract of *M. oleifera* was expressed as percentage. Estimation was conducted in three replicates and mean values are represented.

Sterols

The modified method of Araujo et al., was used with some modification to get the gravimetric weight. The extract of *aq.* drumstick extract of *M. oleifera* was cooled to room temperature at 25°C, filtered on cotton, and the residue (cotton and plant materials) was

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reextracted twice, using 30 mL of chloroform for 15 min. The two filtrates were collected and dried under reduced pressure at 40°C in a rotary evaporator. The residue was then placed in an oven at 80°C until reached constant weight.[25] The quantity of sterols in *aq.* drumstick extract of *M. oleifera* was expressed as percentage. Estimation was conducted in three replicates and mean values are represented.

Tannins

100 mL of distilled water was added to 2g of the *aq.* drumstick extract of *M. oleifera*. The solution was kept in water bath at 90°C for one hour. The mixture was filtered by using Whatman's paper No. 1 and the residue was reextracted again. The two filtrates were collected together and allowed to cooldown. Distilled water was added to the filtrates up to 500 mL. 100 mL of the solution was transferred to a beaker, and then 10 mL of 40% formaldehyde and 5 mL of concentrated H_2SO_4 was added. The whole mixture was refluxed for 30 minutes and was left to cool down. The mixture was filtered, precipitated, dried and the weighed until constant weight. The quantity of tannins in *aq.* drumstick extract of *M. oleifera* was expressed as percentage. Estimation was conducted in three replicates and mean values represented.

Results

The major phytochemicals found in *aq.* drumstick extract of *M. oleifera* were found to be alkaloids, flavonoids, sterols, and tannins. While, the phytochemicals saponins were found to be absent in *aq.* drumstick extract of *M. oleifera* (Table 1).

Table 1: Photochemical analysis of *aq.* drumstick extract of *M. oleifera*

Phytochemicals	***Aq.* Drumstick Extract of *M. oleifera***
Alkaloids	+
Flavonoids	+
Saponins	-
Sterols	+
Tannins	+

The results of quantitative estimation of *aq.* drumstick extract of *M. oleifera* was represented in Table 2 and plotted in Figure 2. Results delineated that tannins were found to be present in highest quantities i.e., 24.89 ± 2.41% in *aq.* drumstick extract of *M. oleifera* followed by flavonoids (6.15 ± 0.28%), sterols (5.04 ± 0.15%), and alkaloids (3.65 ± 0.16%).

Table 2: Quantitative estimation of phytochemicals in *aq.* drumstick extract of *M. oleifera*

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Phytochemicals	**Quantity (%) in Aq. Drumstick Extract of *M. oleifera***
Alkaloids	3.65 ± 0.16
Flavonoids	6.15 ± 0.28
Sterols	5.04 ± 0.15
Tannins	24.89 ± 2.41

Values were expressed as Mean ± SD; n=3

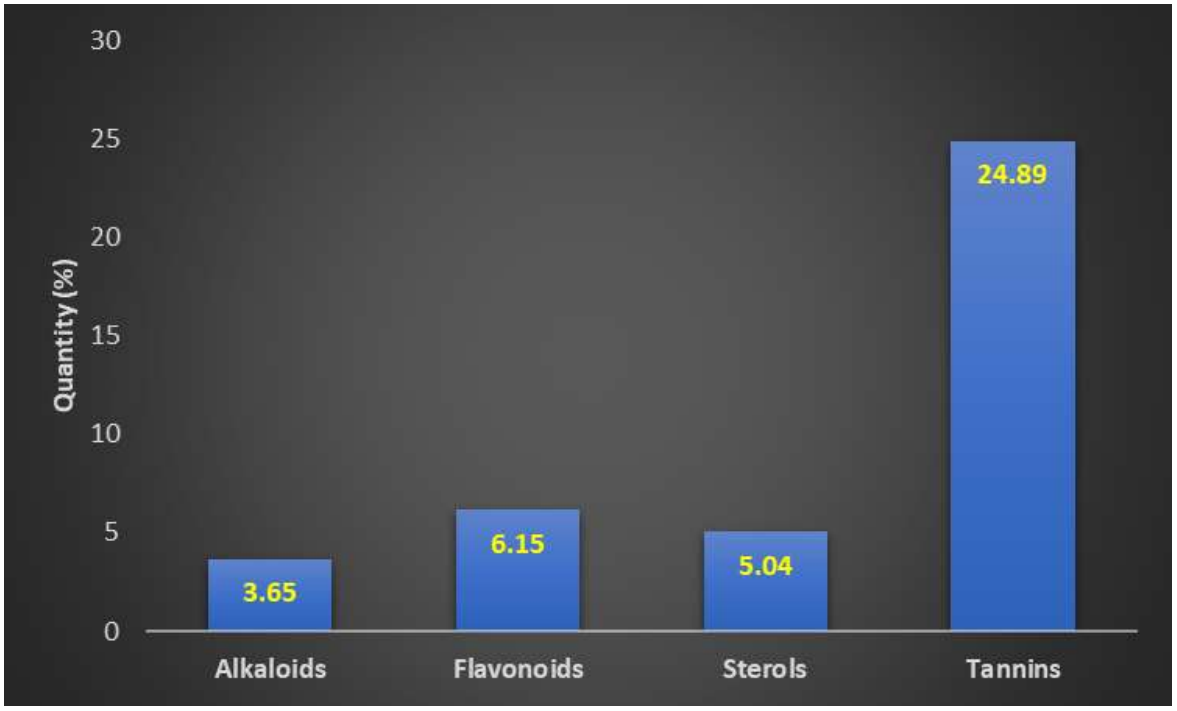

Figure 2: Quantitative estimation of phytochemicals in *aq.* drumstick extract of *M. oleifera*

Discussion

Medicinal plants are an excellent source as they provide a wide variety of possible therapeutic compounds that are both diversified and reasonably safe, compared to manufactured pharmaceuticals.[26,27] According to the World Health Organization (WHO), traditional plant-based medicines constitute the major source of healthcare for more than 80% of the world's population in developing and underprivileged nations.[28,29] The WHO has made an effort to identify all internationally used medicinal plants and recognized over 20,000 species.[30] The demand for plants originated raw materials is increasing at a rate of 15% to 25% annually and is expected to increase by over US$5 trillion by the year 2050.

The estimation of total trade by medicinal plants is approximately US $ 1 billion annually in India.[31] India is incredibly rich in plant species that have therapeutic significance. Most people in society utilize these plants as herbal remedies or as pharmaceutical ingredients in contemporary medicine.[32] Researchers have been concentrating more on herbal

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remedies recently, and various plants are being investigated for potential therapeutic benefits.[33]

Despite the importance of bioactive plant compounds to pharmaceuticals, only a few medicinal plants have been investigated for their phytochemicals and biological activities. The role played by Moringa plant in treatment of several ailments is enormous and a lot of success has been recorded through local use in combating severe illness. There is a need for examination of secondary phytoconstituents that are present in *M. oleifera* plant parts, this will further promote the use of this plant in traditional medicine and will awake our pharmaceutical industries on importance of this plants in treatment of several health challenges.[34] Hence, in the current study we aimed to evaluate the qualitative and quantitative phytochemical analysis of *aq.* drumstick parts of *M. oleifera* plant.

Principally, phytochemical screening or qualitative analysis is used to reveal the chemical constituents or the secondary metabolites of the plant extract or tissue in different plant parts.[35] In our study, qualitative phytochemical analysis of *aq.* drumstick extract of *M. oleifera* revealed alkaloids, flavonoids, sterols, and tannins as major phytochemicals found in *aq.* drumstick extract of *M. oleifera*. Whereas, the phytochemicals saponins were found to be absent in *aq.* drumstick extract of *M. oleifera*. These findings were comparable with literature reports published for qualitative phytochemical analysis of plant extracts by various other research investigators. In a study carried out by Patel et al., aimed to screen phytochemicals present in *M. oleifera* plant. Their study findings revealed the presence of alkaloids, flavonoids, saponins, sterols and tannins in aqueous and ethanolic extracts of leaves of *M. oleifera* plant.[36] Furthermore, Arya et al., reported presence of alkaloids, flavonoids, saponins, sterols and tannins in aqueous, ethanol, ether and chloroform extracts in leaves of *Psidium guajava* L.[37] Gupta et al., reported that in pods of *Acacia concina*, alkaloids were presented in aqueous and methanol extracts but absent in chloroform extract.[38]

Quantitative analysis of secondary metabolites benefits to disclose the chemical constituents of the plant extract and to know which phytochemical conquered over the other. It can also be used to search for bioactive components for developed products that may contain medicinal values and can assist in synthesis of useful drugs.[39] In our study, quantitative phytochemicals estimation of *aq.* drumstick extract of *M. oleifera* revealed that tannins were found to be present in highest quantities i.e., 24.89 ± 2.41% in *aq.* drumstick extract of *M. oleifera* followed by flavonoids, sterols, and alkaloids in 6.15 ± 0.28%, 5.04 ± 0.15%, and 3.65 ± 0.16% respectively. These findings were comparable with literature reports published for quantitative phytochemical analysis of plant extracts by many other research investigators. In *Acacia coccina* pods extract, the quantity of alkaloids, flavonoids, saponins, and tannins was found to be 10.2%, 7.2%, 0.51%, and 2.3% respectively. While in *Acacia catechu*, amount of alkaloids, flavonoids, saponins, and tannins was found to be 11.3%, 0.72%, 0.53%, and 2.1% respectively. In pod extract of *Emblica officinalis*, quantity of alkaloids, flavonoids, saponins, and tannins was found to be 11.2%, 0.04%, 0.55%, and 1.10%.[38]

The findings of our study depicted that Moringa drumsticks contains critical secondary metabolites like alkaloids, flavonoids, sterols, and tannins; especially tannins are

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present in highest quantities in Moringa drumsticks. Literature studies evidenced that phenolic compounds are responsible for blockage of specific enzymes that causes inflammatory disorders. They also protect platelets from clumping through modification of the prostaglandin pathways.[40] As a result of the presence of phenolic compounds i.e., tannins in Moringa drumsticks, could act as antioxidants, anti-clothing agents, immune enhancers, antioxidants, and hormone modulators.[40]

Several functions and roles are attributed to flavonoids in human and animals; this includes protection and fight against inflammatory disorders, allergies, diarrhea, microbes‟ invasion, platelet aggregation, ulcers, hepatotoxins, viruses, and tumors.[41] Flavonoids were able to achieve the aforementioned properties because of their antipyretic, antioxidant, analgesic, and spasmolytic activities.[42] Moreover, presence of epicatechin, quercetin and luteolin in flavonoids plays pivotal roles in inhibition of fluids that is responsible for diarrhea.[42] Hence, Moringa drumsticks could be explored as antipyretic, antioxidant, analgesic, and spasmolytic agents since Moringa drumsticks contains considerable amounts of flavonoids.

Some steroids have cardiotonic activity and some are sex hormones. For example, the steroid hormones, progestogens (progesterone) and the estrogens (estradiol) are female hormones responsible for female sexual characteristics, for the maintenance of pregnancy and for the control of menstrual cycle. Modified estrogens and progestogens are used in oral contraceptives. Androgens (testosterones) are male sexual hormones. Literature studies revealed that plants rich in steroids are used as vegetable for expectant mothers or breast-feeding mothers to ensure their hormonal balance, since steroidal structure could serve as starting material for the synthesis of steroid hormones.[43] Therefore, Moringa drumsticks could be explored in expectant mothers or breast-feeding mothers to ensure their hormonal balance.

Conclusion

In conclusion, the results of our study clearly demonstrated that *aq.* drumstick extract of *M. oleifera* contains secondary metabolites *viz.* alkaloids, flavonoids, sterols, and tannins that are beneficial in treatment of various human ailments. Tannins were found to be present in highest quantities in *aq.* drumstick extract of *M. oleifera.* Hence, Moringa drumsticks could be explored in the traditional medicine as antipyretic, antioxidant, analgesic agents, immune enhancers, and hormone modulators. Further studies for isolation and characterization of secondary phytoactives present in drumstick of *M. oleifera* are recommended for exploitation of these phytoactives in drug formulations.

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