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Research Article

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Qualitative analysis of some bioactive components of methanolic leaf extract of *M. citrifolia* (Noni)

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ABSTRACT

Medicinal plants offer endless opportunities for new drugs discovery due to their supremacy for the possession of phytochemical compounds known for diverse antimicrobial activities. The world ever increasing demand for therapeutic drugs from natural products with particular interest in edible plants for safety purposes is now catching researchers' attention. This study therefore aimed at determining the presence of some bioactive phytochemical components of methanolic leaf extract of *M. citrifolia* L. Qualitative screening of leaf extract has confirmed the existence of Tannins, steroids, saponins, flavonoids and alkaloids in the mixture. And these bioactive compounds correspond to phytochemicals with antimicrobial, nematicide, pesticidal, antioxidant, ant-inflammatory, cytotoxic, anti-allergy, and anti-carcinogenic properties (bioactive compounds) earlier documented by previous researchers.

KEYWORDS: Bioactive compounds, *M. citrifolia*, Methanol, Phytochemicals

INTRODUCTION

The use of synthetic chemical for plant protection is highly effective but the over dependency on these synthetic agrochemicals is predisposing consumers to mycotoxins and build-up of chemical residue in the environment [1]. Medicinal plants are sources of phytochemicals with antimicrobial effect which may serves as safe alternative biopesticide with low toxicity to human and the environment [2]. These phytochemical compounds are plants' defensive/health enhancing compounds, for self-protection against biotic and abiotic stress [3] and [4]. They are regarded as alternative bases for broad spectrum natural biopesticides for the management of plant pathogens, owing to their possession of complex mechanisms of action against pathogenic organisms [5].

According to [6] the world is currently shifting away from the synthetic pesticides to thousands of alternative naturally accuring bioactive compounds, because they are environmentally friendly, broadly effective and safe for both terrestrial and aquatic animals due to low level of acute toxicity. Botanical extracts are normally mixtures of composite bioactive compounds which need to be identified [7].

Qualitative phytochemical screening assays is a vital tool used in bioactive compound analyses, it is inexpensive, quick, and simple, procedure that provides researchers with swift idea of the phytochemical components in a plant extract. This technique uses known and specific tests to prove the presence of a certain compound [8] and [6]. The major steps to identification and utilization of plant base biologically active compound are extraction, screening, and characterization of the compounds, then followed by toxicological assessment. Therefore, this study aimed at extraction and determination of bioactive components; tannins, steroids, saponins, flavonoids, and alkaloids in *M. citrifolia* leaf powder using phytochemical screening assay (chemical tests).

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Fresh *M. citrifolia* leaves were collected around the Universiti Putra Malaysia main library. Collected leaves were identified/confirmed as *M. citrifolia* L. by a botanist at the Herbarium of Biodiversity Unit, Institute of Bioscience (IBS), Universiti Putra Malaysia, (Reference number UPM/IBS/UB/H90/17 and

Voucher number SK 3255/17) (Appendix A). Thereafter leaves were brought to the Biocontrol Laboratory, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia (UPM), washed under running tap water to get rid of dust and debris and rinsed in sterile distilled water. Leaves were first allowed to stay for 6 hrs under the laminar air flow to dry up the wet leaves surface. And finally dried at 40- 45°C in a mechanical convection oven (Memmert, Germany). An electrical grinder Retsch SK100 standard Guβeisen, 2002 was used to gried the dried leaves into the powdered form for use [5].

Test for Tannins

To determine the presence of Tannins, Braemer's test was performed. Following the method described by [6], where 2 g of the powdered *M. citrifolia* leaf was dissolved in 10 ml of methanol, then macerated and filtered by means of cotton wool and funnel. Thereafter, 2 ml of the filtrate was added to 2 ml of 10 % alcoholic ferric chloride (1:1). Formation of greenish grey coloration of the mixture indicates the presence of tannins.

Test for Steroids

To test for the presence of steroids, Lieberman Burchardt test was used. For the test, 2 g of the leaf powder was added to 20 ml of methanol in a 150 ml conical flask and covered for 30 min, mixture was filtered using cotton wool and funnel. Filtrate was poured into a 50 ml beaker and placed on a water bath until filtrate was completely evaporated. 6 ml of chloroform was added to the evaporated extract and mixed thoroughly. Then 2 ml of the chloroform mixture was transferred into a test tube where few drops of acetic anhydrite was added, followed by addition of few drops of H_2SO_4, which was added slowly to the wall of the test tube. Formation of dark green colour designated the presence of steroids [9]. Plants steroids are derivatives of cyclization of the triterpene squalene [10] and [11].

Test for Saponins

To determine the presence of saponin in the phytochemical components of *M. citrifolia* leaf. 70 ml of sterile distilled water was placed in a beaker containing 3 g of plant powdered leaf, mixed then boiled for 2 min. Mixture was filtered into a new test tube using cotton wool and funnel to produce an aqueous extract. 2 ml of the aqueous extract was discharged into a graduated test tube, and vigorously agitated. The formation of 1cm form that persists for 3 minutes designated the presence of saponins [12].

Test for Flavonoid

Ammonium test was employed to test for the presence of Flavonoid in leaf extract of *M. citrifolia*, following the method as described by [13] and [14]. To achieve this, 0.2 g of *M. citrifolia* leaf powder was added to 10 ml of ethyl acetate in a 100 ml conical flask. Mixture was then heated for 5 min in a water bath, allowed to cool and filtered. 4 ml of the filtrate was discharged into a test tube where 1 ml of diluted ammonia solution was added to the mixture, agitated and kept at room temperature for a few seconds then observed. Appearance of layer of yellow coloration at the bottom of the test tube indicates the presence of flavonoid

Test for Alkaloids

Dragendroff reagent test: For this test, 0.2 g of *M. citrifolia* leaf powder was added to 20 ml of diluted H_2SO_4 in methanol in a conical flask. Mixture was boiled for 5 minutes in a water bath, cool and filtered. Three drops of dragendroff reagent were added to the filtrate. Formation of creamy, orange solution indicates the presence of alkaloids [15].

RESULTS AND DISCUSSION

The qualitative analysis of phytochemical components of *M. citrifolia* leaf extract using the conventional phytochemical screening assay (chemical tests) detected the presence of tannins, steroids, saponins, flavonoids, and alkaloids (Table 1 and Figure 1) which is in agreement with the findings of [16] and [17]. During the Braemer's test, the *M. citrifolia* leaf extract turned greenish grey which was an indication for the presence of Tannins in the solution according to the reports of [18] and [8]. Tannins belong to the class polyphenol compounds with an astringent property, soluble in acetone, alcohol, and water. Similarly, Lieberman test (Burchardt test) of the leaf extract solution turned dark green in colour (Figure 2) which was an indication for the presence of steroids. This finding is in line with the report of [8]. The Frothing test also showed the formation of I cm form height above the mixture which persisted for more than 3 min. According to [6], the appearance of up to 1 cm form height above mixture that lasts for up to 2-3 minutes is an indication for the presence of saponins (Figure 3). Saponins are naturally produce by many plants for defense against pest and pathogens, they are easily converted to drugs, cosmetics and taste modifiers and are therefore considered economically viable compounds [19] and [20]. Result for the Ammonium test (test for flavonoids) showed layer of yellow coloration at the bottom of the test tub, which is an indication for the presence of flavonoid in the leaf extract, according to [21] and [14] (Figure 4). Flavonoids is another member of the compounds class polyphenols which are known for their Pesticidal, antimicrobial, antioxidant, chemotherapy activities, and their mechanism of action against microorganisms includes; complex activities with the cell wall, cell lysis, membrane disruption, inactivation of enzymes and death [22]. Findings of the Dragendroff test (test

Table 1: Phytochemical constituents of m. Citrifolia leaf powder

Phytochemical compound	Chemical test	Indicator	Result
Tannins	Braemer's test	Greenish grey	+
Steroids	Burchardt test	Dark green colour	+
Saponins	Froth test	1 cm form that lasted for 3 minutes,	+
Flavonoids	Ammonium test	A layer of yellow coloration at the bottom	+
Alkaloids	Dragendroff test	Creamy orange coloration	+

Key: (+) indicates the presence of a phytochemical compound

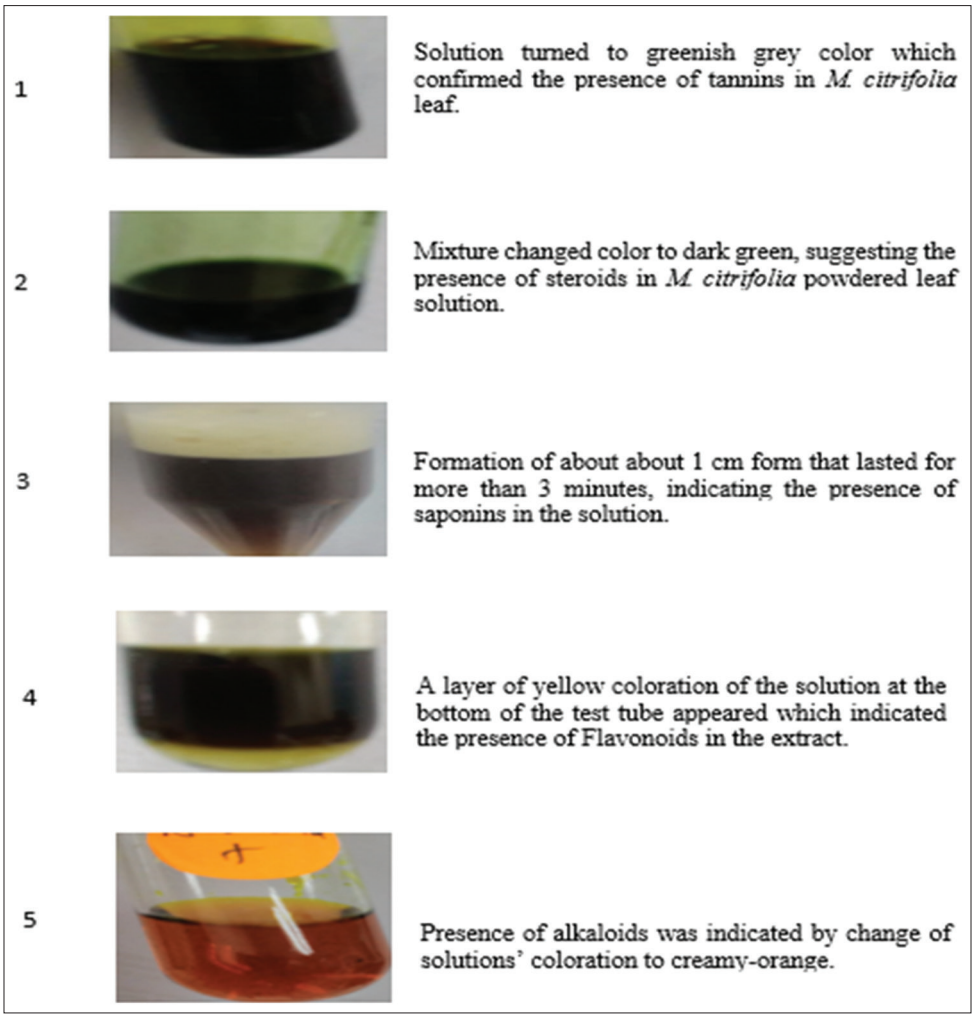

Figure 1: Pictorial summary of phytochemical screening assay tests for tannins steroids, saponins, flavonoids and alkaloids components of *M. citrifolia* leaf extract.

for Alkaloids) shows formation of layer of creamy-orange solution (Figure 5). This result is in agreement with the report of [15], that by this test, the formation of creamy-orange colouration of the test solutions suggests the presence of alkaloids in the test solution. Alkaloids have a toxic effect on microbials, in human medication and or as biopesticides [22].

CONCLUSION

The methanolic leaf extract of *M. citrifolia* has the following significant/potent bioactive compounds; tannins, steroids, saponins, flavonoids and alkaloids. These compounds also correspond to those with antimicrobial antioxidant, pesticidal, anticancer, ant-inflammatory, Hepatoprotective activities earlier reported by [23] and [7]. Therefore, *M. citrifolia* leaf extract can be utilise as a new source of drugs and pesticides for both agrochemical industries and pharmaceutical industries.

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