



doi: <http://dx.doi.org/10.19240/njpas.2019.A06>

Qualitative and Quantitative Phytochemical analysis of *Maerua crassifolia* leaves using various solvents

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ABSTRACT

The determination of biologically active components from botanical origin is mainly dependent on the type of solvent used. Qualitative and quantitative phytochemical screening of different solvent extracts of *Maerua crassifolia* leaf was carried out using standard methods. Ethanol, chloroform, methanol, deionized water, acetone and petroleum ether were used as solvents. The presence of eight secondary metabolites (alkaloid, saponin, flavonoid, cardiac glycosides, tannin, phenols, anthraquinones and steroids) was tested. The results showed that four of these phytochemicals were present in all the solvent extracts (alkaloid, flavonoid, phenol, and combined anthraquinones) while, cardiac glycoside was not detected in any of the extracts. Although saponin was present in only four of the solvent extracts, it had the highest value in the quantitative analysis (120.37 ± 0.18 mg/100g) from the aqueous extract while the highest quantity of flavonoids (38.47 ± 0.32 mg/100g) was from the acetone extract. Percentage alkaloids ranged between (0.025 ± 0.007 mg/100g) from the methanol extract to (0.93 ± 0.03 mg/100g) from the aqueous extract. Petroleum ether extracted the highest quantity of combined anthraquinone (74.61 ± 0.22 mg/100g). The presence of various phytochemicals has further established the previous findings that *Maerua crassifolia* leaf is a good source of therapeutic compounds. The present study reveals saponins as the most prominent phytochemical components of *Maerua crassifolia* leaf extracted using the solvents under study and further identifies deionized water as the most appropriate solvent for the extraction of the saponins from the leaves.

Keywords: Qualitative, Quantitative, Phytochemical analysis, *Maerua crassifolia*

Introduction

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which shield plant cells from environmental hazards such as pollution, strain, drought, UV exposure and pathogenic attack (Ali and Alqurainy, 2006). They are found in fruits, vegetables, grains, nuts, seeds,

flowers, leaves and roots (Gemede, and Ratta, 2014), promote human health (Nyamai *et al.*, 2015) and prevent diseases (Ekwueme *et al.*, 2015; Nyamai *et al.*, 2015). In the past few years, these compounds have been established to be beneficial in the cure of

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various ailments especially in humans (Gemedede, and Ratta, 2014).

Maerua crassifolia Forssk. (*Capparaceae*) is a small evergreen tree, which thrives in the dry savannas and the semi-desert regions of sub Saharan Africa widely used in ethnomedicine for the treatment of different diseases such as malaria (Akuodor *et al.*, 2014a) skin diseases (Saad and Asalam, 2015). The application of *M. crassifolia* leaves in ethnomedicine is not a coincidence as studies (Akuodor *et al.*, 2014a; Akuodor *et al.*, 2014b) have detected many phytochemicals in these leaves. Regrettably, there is a dearth of information on the quantitative determination of these phytochemicals in the leaves. The effective extraction, determination as well as isolation of biologically active components from plant material have been known to be largely dependent on the type of solvent used in the extraction procedure (Tiwari *et al.*, 2011). There is therefore a need to carry out the quantitative as well as the qualitative analysis of the phytochemicals present in *M. crassifolia* leaves using various solvent.

MATERIALS AND METHOD

Sample Collection and identification

Fresh leaves of *M. crassifolia* were obtained from Shagari local government, Sokoto, Sokoto state, Nigeria. The sample was identified at the Botany Unit of the Department of Biological Science, Faculty of Science, Usman Dan Fodio University, Nigeria with an identification voucher number UDUH/ANS/0210.

Sample preparation

The fresh leaves of *M. crassifolia* were properly cleaned to remove any dirt or filthy particles present on the surface, washed carefully with deionized water and air dried under shade. The dried plant materials were taken separately and crushed using an electric blender to obtain a fine powder and sieved (2mm mesh) to obtain finer particles. The powdered

samples were packaged in a polyethylene bag where they were stored until needed for analysis.

Extraction Solvents and Method

Chloroform, acetone, petroleum ether, methanol, ethanol and deionized water were used as solvents for the study, while maceration was the method of extraction employed.

Phytochemical screening

Procedure

Ten gram (10 g) portion of the powdered leaves was weighed and soaked in 150 ml of each solvent at room temperature for 48 hours with vigorous shaking at regular interval. The materials were filtered first with muslin cloth and then with filter paper (Handa *et al.*, 2008).

By means of standard procedures, the filtrate was used for the qualitative and quantitative screening of the following phytochemicals; alkaloids, tannins, saponins, flavonoids, steroids, phenols, cardiac glycosides and combined anthraquinones.

Qualitative tests

Test for Tannins (Braymer's test)

One gram (1 g) of the sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration (Sethupandian *et al.*, 2016).

Test for Alkaloids (Dragendorff's test)

A quantity of 0.5 ml of extract was treated with 4ml of 1% HCl and boiled in water for 5 minutes and later filtered. 1ml of the filtrate was treated with 2-3 drops of Dragendorff's reagent. Turbidity shows the presence of alkaloid (Sethupandian *et al.*, 2016).

Test for Flavonoids

One millilitre (1 ml) of extract was treated with 2 ml of dilute NH_3 (1%) and small quantity of concentrated H_2SO_4 was added. Yellow coloration would be observed but disappears on standing in the presence of flavonoids (Yadav *et al.*, 2014).

Test for Saponins

One millilitre (1ml) of extract was boiled with 10ml of distilled H₂O for 5mins and later filtered. 5 ml of the filtrate was added to 5ml of water and shaken vigorously. This was further mixed with 3 drops of olive oil. Shake again for the formation of emulsion. This indicates the presence of saponins (Yadav *et al.*, 2014).

Test for Steroids

A volume of 1 ml of extract was treated with 1 ml of acetic anhydride and few drops of H₂SO₄. The colour changes from violet to blue green in the presence of steroids (Yadav *et al.*, 2014).

Test for Combined Anthraquinones

A volume 1 ml of extract was boiled with 3 ml of FeCl₃ and 3 ml of 1% HCl. The solution was filtered and shaken. 3 ml of benzene was added to the solution. The benzene layer was removed and equal volume of 10% NH₃ was added to the solution. The mixture was shaken vigorously and observed for the formation of pink colour (Harbone, 1999).

Test for Cardiac Glycosides

A volume of 0.1 ml of extract was treated with 1 ml of glacial acetic acid and 1 drop of 3% FeCl₃ underlayered with 1 ml of H₂SO₄. Formation of brown tiny ring at interphase indicates the presence of cardiac glycosides (Harbone, 1999).

Test for Phenols

A volume of 1 ml of extract was treated with 2 ml of FeCl₃ solution and observed for deep blue or green colour formation (Harbone, 1999).

Quantitative Tests**Determination of Total Tannins**

A volume of 1 ml of the sample was added to 10 ml of distilled water, incubated for 1 hour and then filtered. The volume of the solution was increased to 25 ml with distilled H₂O. A quantity of 4 drops of Tannin reagent was added to 1 ml of the filtrate and 5 ml of distilled water. The absorbance was read

after 10 minutes at 720 nm using Tannin as a standard (Nyamai *et al.*, 2015).

Estimation of Alkaloids (Harborne Method)

An aliquot of 1 ml of the sample was added to 20 ml of 10% acetic acid in ethanol. The mixture was covered and allowed to stand for 4 hours and then filtered. The filtrate was concentrated on a water bath to reduce a quarter of its original volume. Concentrated NH₄OH was added to the filtrate and the precipitate was collected through weighed filter paper. This was followed by rinsing the precipitate with 1% NH₄OH and then filtered. The alkaloid precipitate was dried in an oven at 60°C for 30 minutes and the filter paper was reweighed. (Harborne, 1999) The alkaloid content was calculated in percentage as:

$$\% \text{ Alkaloid} = \frac{\text{Final Weight of sample}}{\text{Weight of sample}} \times \frac{100}{1}$$

Total Flavonoid content estimation

An aliquot of 1 ml of sample was added to 4 ml of water which was left to stand for 5 minutes. 0.3 ml of 5% Sodium nitrite and 0.3 ml of 10% AlCl₃ were added to the mixture and later incubated for 6 minutes at room temperature. After heating, 2 ml of NaOH was added to the incubated mixture and the volume was made to increase to 10 ml with distilled H₂O. The absorbance was measured at 510 nm. Quercetin was used as a standard against a blank (0.01g of Quercetin in 100 ml of H₂O) (Vijay and Rajendra, 2014).

Estimation of Saponins

The method Obadoni and Ochuko (2001) was adapted. A quantity of 1 g of extract was treated with 25 ml of 20 % Ethanol and this mixture was heated for in a water bath for 2 h with continuous stirring at

about 55°C. The extracted residue was added to 50 ml of 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 °C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight.

Estimation of Steroid content

An aliquot of 2 ml was taken from the powdered extract prepared in 50 ml of distilled water and was shaken for 1 hour. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at $70 \pm 2^\circ \text{C}$ for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank (Narendra, *et al.*, 2013).

Estimation of Combined Anthraquinones

A volume of 1ml of sample was added to 5ml of H₂O. The mixture was boiled for 1 hour, filtered and 5ml of 50% Ethanol was added. The mixture was later filtered again. The absorbance was read at 450nm.

Estimation of Total Phenolic Compound

A quantity of 0.5g of the extract was weighed and dissolved in 50 ml of water. A portion of 0.5 ml was added to 0.1ml of Folin C (Folin- Ciocalteu) reagent. It was mixed and incubated at room temperature for 15 minutes. After which 2.5 ml of sodium carbonate solution (7.5% w/v of Na₂CO₃) was added and further incubated for another 30 minutes at room temperature. The absorbance of the solution was measured at 760 nm with the use of Gallic acid as standard. The concentration of total phenol was expressed as Gallic acid equivalent (GAE) (mg/g of dry mass) which is a commonly used reference value (Nyamai *et al.*, 2015).

RESULTS

The result of this study revealed the presence of alkaloids, flavonoids, phenols and combined anthraquinones in the extracts of the six solvents (acetone, chloroform, ethanol, methanol, petroleum ether and deionized water) used in this study. Tannins, saponins and steroids were found in most but not all the solvent extract used. Cardiac glycosides were however absent in all the solvent extracts studied. These results are presented in Table 1. The results of the quantitative analysis revealed that saponins were the most present phytochemicals in the solvent extracts. The highest level of saponins (120.37±0.18 mg/100g) was found in the deionized water extract. The level of the phytochemicals present in the different extracts of *M. crassifolia* leaves have been presented in Table 2.

Table 1: Phytoconstituents of different extracts of *Maerua crassifolia* leaf.

Phytoconstituents	Acetone	Chloroform	Ethanol	Methanol	Pet. Ether	Deionized Water
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Combined. Anthraquinones	+	+	+	+	+	+
Tannins	+	-	+	+	+	+
Saponins	+	-	+	+	-	+
Steroids	+	+	-	+	+	-
Cardiac glycosides	-	-	-	-	-	-

+ indicates presence

- indicates absence

Table 2: Results of the quantitative analysis *Maerua crassifolia* leaf extracts

	Acetone	Chloroform	Ethanol	Methanol	Pet.Ether	Deionized water
Phytoconstituents (mg/100g)						
Alkaloids (%)	0.04±0.000	0.20±0.007	0.095±0.007	0.03±0.007	0.3±0.014	0.93±0.350
Flavonoids	38.44±0.32	20.39±0.20	38.24±0.25	17.49±0.19	24.52±0.13	12.67±0.20
Phenols	21.86±0.11	18.78±0.16	32.05±0.16	27.29±0.23	16.09±0.22	38.02±0.11
Combined Anthraquinones	34.82±0.28	29.34±0.23	48.07±0.17	11.99±0.23	74.61±0.22	67.98±0.23
Steroids	39.40±0.28	37.40±0.02	-	27.12±0.02	10.05±0.07	-
Saponins	106.83±0.37	-	97.55±0.47	107.10±0.56	-	120.37±0.18
Tannins	49.48±0.34	-	31.98±0.18	24.37±0.35	20.45±0.10	15.08±0.18
Cardiac Glycosides	-	-	-	-	-	-

Values represent Mean ± standard deviation of duplicate determination

Except for Alkaloids all phytoconstituents are in mg/100g

Discussion

Quantitative and qualitative phytochemical screening of various solvent extracts of *Maerua crassifolia* leaf has been studied using acetone, chloroform, ethanol, methanol, petroleum ether and deionized water.

Qualitative Analysis

Alkaloids, flavonoids, combined anthraquinones and phenols were present in all of the solvent extracts of the leaf studied. Except for anthraquinones, the presence of these phytoconstituents are in agreement with those recorded by Chilaka *et al.*, (2015) in the methanol leaf extract of *M. crassifolia*. In a similar study using *Calotropis procera* leaves, Abegunde and Ayodele-Oduola (2013) observed the presence of alkaloids in the ethanol extract of the leaves and all the solvents used in this study, but not in the petroleum ether contrary to the results in this study. This may be attributed to the difference in the test methods used in both studies (Fehli *et al.* 2017). All the solvents were also positive for tannins except chloroform. This agrees with the finding of Tiwari *et al.* (2011) who noted that tannins are better extracted with more polar solvents as was also the case with saponins.

Saponins were not detected in the chloroform and petroleum ether extracts. Steroids were not observed in the ethanol and aqueous extracts this may not be unconnected with the hydrophilic nature of steroids (Tiwari *et al.*, 2011). Cardiac glycosides were however absent in all the solvent extracts studied; this is in contrast with the report of Chilaka *et al.*, (2015) and could be attributed to the differences in the method of extraction (Fehli *et al.* 2017). The presence of these phytoconstituents in all the solvent extracts studied show that *Maerua crassifolia* has good potential as a medicinal plant (Nostro, 2000). Studies have shown varying phytoconstituent presence in various plant parts and extracts (Abegunde and Ayodele-Oduola, 2013; Chilaka

et al., 2015; Ekwueme *et al.*, 2015), the proper harnessing of these nature's gifts will depend not only on the qualitative screening but also on the ability to quantify these phytochemicals.

Quantitative Analysis

The quantitative analysis of the solvent extracts revealed that the level of saponins in *M. crassifolia* leaf was higher than the other phytochemicals studied. The aqueous (deionized water) extract recorded the highest level of saponins (120.37 ± 0.18 mg/100g) the methanol, acetone and ethanol extracts were equally appreciable when compared with the level of other phytochemicals present in the leaves. This high level of saponins in these extracts agrees with the result of Ramya and Dhamotharan (2015) and like the present study saponins was not detected in the chloroform and petroleum. Saponins are known to have hypocholesterolaemic (Chung, 2004), immunostimulant (Guruvayoorappan, *et al.*, 2015), anticarcinogenic (Dastmalchi *et al.*, 2007) and antidiarrhoeal properties (Tiwari *et al.*, 2011). The presence of such high levels of saponins in comparison with other phytochemicals present in the *M. crassifolia* leaves therefore confirms the profound antidiarrhoeal property of the methanolic extract of the leaves recorded by Akudor *et al.* (2014b).

Combined anthraquinones were also found to be next in quantity after saponins with the highest level (74.61 ± 0.22 mg/100 g) found in the petroleum ether extract then the aqueous extract (67.98 ± 0.23 mg/100 g). This shows that petroleum ether will be the best among all the solvents for the extraction of combined anthraquinones. Anthraquinones constitute an important class of natural and synthetic compounds with a wide range of applications and have been found medical applications in the alleviation of constipation, arthritis, multiple sclerosis, and cancer (Malik and Muller, 2016).

The alkaloid level in the various extracts ranged between $0.03 \pm 0.007\%$ in the methanol extract and

0.93±0.350% in the aqueous extract. Although alkaloids were detected in all the extract studied, it was obvious that deionized water extracted the highest quantity of alkaloids. However, *Vernonia amygdalina* leaves having 1.34% (Okeke *et al.*, 2015) have a higher level of alkaloids. Apart from having antidiarrhoeal properties, alkaloids also have antihelmintic properties (Tiwari *et al.*, 2010).

The *M. crassifolia* leaf showed more tannin content when extracted with acetone (49.48 ± 0.34 mg/100g) than ethanol (31.98 ± 0.18 mg/100 g), methanol (24.37 ± 0.35 mg/100 g), petroleum ether (20.45 ± 0.10 mg/100 g) and deionized water (15.08 ± 0.18 mg/100 g) which showed the least tannins content with zero extraction in the case of chloroform. Like saponins and alkaloids, tannins also have antidiarrhoeal properties (Tiwari *et al.*, 2010).

Steroids showed a better extraction with acetone (39.40 ± 0.28 mg/100 g) followed by chloroform (37.40 ± 0.02 mg/100 g), methanol (27.12 ± 0.02 mg/100 g) then petroleum ether (10.05 ± 0.07 mg/100 g) with no results with ethanol and deionized water. This is contrary to the result of Ramya and Dhamotharan (2015) who were able to detect steroids from ethanoic and aqueous extract *Costus speciosus* of the leaves.

Maximum extraction of flavonoids (38.44 ± 0.32 mg/100 g) in the study was obtained with acetone while deionized water extracted the least quantity of flavonoids (12.67 ± 0.20 mg/100 g)

The deionized water extract showed maximum content of phenols (38.02 ± 0.11 mg/100 g), while petroleum ether extracted the least amount (16.09 ± 0.22 mg/100 g).

The presence of these phytochemicals authenticates the use of *M. crassifolia* in ethnomedicine for the cure of various ailments. Akuodor *et al.* (2014a), Akuodor *et al.* (2014b) and Akuodor *et al.* (2015), have verified some of these claims using the methanol extract which was found to exhibit antimalarial, antidiarrhoeal,

analgesic, anti-inflammatory, antipyretic and gastrointestinal activities.

Conclusion

This study has shown that *M. crassifolia* leaves contain an appreciable amount of saponins which can be readily available in the aqueous, methanol and acetone extract. Chloroform is not a good solvent for the extraction of saponins and tannins from these leaves. Finally, alkaloids, flavonoids, phenols and anthraquinones were detected in varying quantities in all the solvents extracts used in this study.

Acknowledgement

The authors are grateful to the Botany Unit of the Department of Biological Science, Faculty of Science, Usman Dan Fodio University, Nigeria and Mr. Sunday Adenekan of Biolife Consults for their technical assistance.

Conflict of interest

The authors declare that there is no conflict of interest.

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