Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
JMPS 2019; 7(2): 21-24

Received: 14-01-2019
Accepted: 18-02-2019

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The antimicrobial activity of the leaves of *Urtica massaica* on *Staphylococcus aureus*, *Escherichia coli*.

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Abstract
Medicinal plants have great potential for providing novel drug leads with novel mechanism of action. The present study aimed at determining the antimicrobial activity and phytochemical profile of methanol and aqueous extracts of *Urtica massaica* against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method. Inhibition zones of aqueous crude extracts of *U. massaica* arraigned between 6.50 and 6.67mm while those of methanolic extracts were between 6.33 and 8.42mm. The aqueous crude extracts of *U. massaica* did not show any antimicrobial activity against *E. coli.* The methanolic extract of the *U. massaica* showed presence of alkaloids, saponins, tarpenoids, steroids and flavonoids. It was concluded that the methanolic extracts of *U. massaica* could be potential source of drug formulation against the *S. aureus* and *E. coli*.

Keywords: Urtica massaica, microbial pathogen, extracts, antimicrobial activity

Introduction
Emerging multidrug resistance by many microorganisms calls for exploration of new sources of drugs alternatives [1, 2]. Plants are largely unexplored source of drug repository [3, 4]. Medicinal plants have great potential for providing novel drug leads with novel mechanism of action [5-7]. Therefore, the present study aimed at determining the antimicrobial activity and phytochemical profile of methanol and aqueous extracts of *Urtica massaica* against clinical isolates of *Staphylococcus aureus* and *Escherichia coli* using bioassay testing of crude extracts at different concentrations. The antimicrobial activity of the extracts was determined by measuring the zones of inhibition using the disc diffusion method [8, 9]. Significant differences in inhibition zones between the crude extracts of *U. massaica* and positive control (Gentamycin and Ciprofloxacin) were reported against the selected group of microorganisms. However, non-significant differences in inhibition zones between aqueous and methanolic crude extracts of *U. massaica* were reported *S. aureus.* Inhibition zones of aqueous crude extracts of *U. massaica* arraigned between 6.50 and 6.67mm while those of methanolic extracts were between 6.33mm and 8.42mm.The aqueous crude extracts of *U. massaica* did not show any antimicrobial activity (6.00mm) against *E. coli.* The methanolic extract of the *U. massaica* showed presence of alkaloids, saponins, tarpenoids, steroids and flavonoids. It was concluded that the methanolic extracts of *U. massaica* could be potential source of drug formulation against the *S. aureus* and *E. coli.*

Materials and Methods
Preparation of Plant material
Urtica massaica leaves were collected on the basis of indigenous knowledge from Eldoret, Kericho, Kitale and Marigat, Kenya. Taxonomical identification was done at University of Eldoret herbarium by a botanist and voucher specimen deposited. The plant material was ground into fine powder, weighed and stored for other subsequent procedures that followed [10].

Extraction
Crude extracts from the stored fine powder were prepared using hot water and methanol. The crude extracts were later to be reconstituted to attain the desired concentration was prepared by dissolving 50 g of the ground samples into 100 ml of the respective solvents [11].

Ethanol extraction

Fifty grams of the ground plant parts were placed in a 250 ml conical flask and 100 ml of 70% ethanol added into the flask and shaken well. The mixture was left to settle for 24 hours then filtered. The filtrate was transferred into a round-bottomed flask, attached to a rotary evaporator until the ethanol evaporated leaving a thick paste and then transferred to a vial and left to dry in front of a fan until a solid was obtained indicating that all the ethanol had evaporated from the paste. The solid was stored in a refrigerator at 2^0C - 4^0C for other processes that followed.

Aqueous extraction

50 grams of the ground samples were mixed with 100 ml of distilled water in a 1 litre conical flask and the mixture shaken until completely dissolved. The flask was placed in a shaking water bath at 70±1 ^{0}C for one and a half hours. The mixture was removed and filtered using surgical cotton wool in a glass funnel and left to cool and transferred into a 250 ml round bottom flask. The filtrate was put in a round-bottomed flask then inserted into a shallow tray containing acetone and dry ice to freeze dry, and coat on the flask. The sample was freeze-dried using Modulyo K4 freeze dryer (EDWARDS) so as to completely eliminate any water through vacuum until it was completely dry. The dried sample was removed from the flask, weighed into vials and refrigerated at 2 - 4 ^{0}C.

Evaluation of Antimicrobial activity

Clinical isolates of *Staphylococcus aureus* and *Escherichia coli* were used in bioassay testing using crude extracts at different concentrations. Positive controls for antibacterial drugs were Cyprofloxacin and Gentamicin respectively [12]. The test crude extracts was reconstituted using organic solvents (DMSO) and sterile distilled water necessary to ensure that the solvents used for extraction and dissolution would not have inhibitory actions. All experiments were carried out in triplicates [13].

Antimicrobial assays

Bacteria used in the current study included the Gram positive; *Staphylococcus aureus* while Gram negative *Escherichia coli* which were obtained from the KEMRI Centre for Microbiology Research, Nairobi. The Gram positive bacteria used were *Staphylococcus aureus* ATCC 2593 while Gram negative are *Escherichia coli* ATCC 27853. Preparation for bioassays was done by sub-culturing the bacterial strains were sub-cultured on Mueller Hinton agar at 37 ^{0}C for 24 hours to obtain freshly growing strains [14]. The antimicrobial activity of the extracts was determined by measuring the zones of inhibition using the disc diffusion method [15].

Approximately 100 mg sample of each extract was dissolved in 1ml of each solvent to produce 100 mg/ml. Approximately 20 µl of each preparation was then measured and impregnated on to 6 mm filter paper discs prepared from Whatman No 1 [16]. These were sterilized in an autoclave purposely for disc diffusion assay and allowed to air dry. The disks were then placed aseptically onto the inoculated plates and incubated for 24 hours at 37 ºC. After incubation, the inhibition zone diameters were measured in millimeters and recorded against the corresponding concentrations [17, 18]. Positive controls were set against standard antibiotics. Discs of Ciprofloxacin, and Gentamycin (25µg) were used as standards the positive control for bacteria. Discs containing 70% DMSO were used as negative controls. Inhibition zone diameters were expressed as mean inhibition zones of the three replicated assays. Classification of the antimicrobial activity was stipulated as ranging from little or no activity at ≤ 10 mm to very strong activity for inhibition zone diameters of ≥ 30 mm.

Determination of minimum inhibitory concentration (MIC)

Disk diffusion method was used to determine the minimum inhibitory concentration of the active crude extracts against the test microorganisms [19]. The tests were performed in well-micro-titer plates where the plant extracts were dissolved in respective solvents then transferred into micro-titer plates to make serial dilutions ranging from 10^1 - 10^{10}. The final volume in each well was 100 µl and the wells were inoculated with 5µl of microbial suspension. The bacteria were incubated at 37 ^{0}C for 24 hours. The MIC value was determined as the lowest concentration of crude extract in broth medium that inhibited visible growth of test microorganism as compared to the control where Dimethylsulphoxide (2 drops) dissolved in water [20]. The MIC was recorded as the lowest extract concentration demonstrating no visible growth as compared to the control broth turbidity. Wells that were not inoculated were set to act as controls for the experiment and the experiments were done in triplicates whereby average results were recorded [21].

Phytochemical Screening

Phytochemical screening was done on the active extracts to identify the phytocompounds present. The phytochemical constituents of the different extracts were separated by thin layer chromatography (Kieselgel 60 F254 0.2 mm, Merck) [22]. Thin layers chromatography (TLC) plates were developed with Ethyl acetate: petroleum spirit (3:7) as the solvent system for dichloromethane extracts while dichloromethane: methanol (9.5: 0.5) solvent system was utilized for methanol extracts. The separated constituents on a silica gel plate were visualized under ultra violet light (254 nm and 365 nm) then sprayed with visualizing agents for colorimetric view [23, 24].

Results

Antimicrobial activity of *U. massaica*

Results on the effect of methanolic and aqueous crude extract of *U. massaica* on selected groups of microorganisms are presented in Table 1. Significant differences (P<0.05) in inhibition zones between the crude extracts of *U. massaica* and the positive control (Gentamycin and Ciprofloxacin) were reported against the selected group of microorganisms. However, non-significant differences (P>0.05) in inhibition zones between aqueous and methanolic crude extracts of *U. massaica* were reported among the two microorganisms. The aqueous crude extracts of *U. massaica* showed lowest inhibition zone of 6.67 mm against *S. aureus*. However, this was not significantly different from 8.42mm for the methanolic extract. Significant highest inhibition zones of 21.00 mm and 20.00 mm against *S. aureus* were recorded in plates with Gentamicin and Ciprofloxacin respectively.

Table 1: Effect of solvents/controls on growth activity of selected group of microorganisms

Solvent/Controls	*Staphylococcus aureus*	*Escherichia coli*
Aqueous	6.50±0.67a	6.00±0.00a
Methanol	8.42±2.25a	6.33±0.49a
Gentamycin	21.00±0.00c	22.00±0.00c
Ciprofloxacin	20.00±0.00b	21.00±0.00b
F-Value	508.42	15550.00
P-Value	0.00**	0.00**

Means followed by different letters within a column are significantly different at P<0.05

**denotes significance at P<0.05

The aqueous crude extracts of *U. massaica* did not show any antimicrobial activity (6.00mm) against *E. coli* while methanoic crude extracts recorded minimum inhibition zone of 6.33mm. Significant hight inhibitions against *E. coli* were recorded in Gentamicin (21mm) and Ciprofloxacin (20mm). The growth inhibitions (mm) of aqueous and methanolic crude extracts against *A. flavus* were 6.67 and 7.58 respectively, which were significantly different from those of positive controls (Gentamicin 19.00mm and Ciprofloxacin 20mm). In addition, aqueous and methanolic crude extracts of *U. massaica* were found to be not significantly different (p>0.05), with inhibition zones of 6.58mm and 6.92mm against *C. albicans* respectively. However, these were shown to be significantly lower from those Gentamicin (19mm) and Ciprofloxacin (20mm) as indicated in Table 1.

Minimum Inhibition Concentration

Table 2 shows Minimum inhibitory concentration in mg/ml of *U. massaica* extracts against the various pathogens. MICs were done on extracts that showed activity. Generally, *U. massaica* proved to be more active against *S. aureus*. The MIC results for *U. massaica* ranged from 0.1 mg/ml to 1000 mg/ml against the test isolates. The methanolic extracts were most active on different isolates with MIC value of 0.1 mg/ml to 100 mg/ml against *S. aureus*.

Table 2: Results of MIC of the plant and different solvents in mg/ml

Plant	Region	Test sample (solvent)	Test organism	MIC (mg/ml)
U. massaica	Marigat	Water	*S. aureus*	100
U. massaica	Kericho	Methanol	*S. aureus*	0.1
U. massaica	Marigat	Methanol	*S. aureus*	10
U. massaica	Eldoret	Methanol	*S. aureus*	100

Phytochemical analysis of *U. massaica*

Results on phytochemical analysis of *U. massaica* are shown in Table 3. A wide range of various phytochemicals; alkaloids, glycosides, saponins, tarpenoids, phenols, steroids, tannins and flavonoids were tested with their appropriate protocols and reagents. The *U. massaica* methanolic extracts showed presence of most of the phytochemicals tested.

The methanolic extract of the *U. massaica* from Eldoret showed the presence of saponins, tarpenoids, steroids and flavonoids in high levels as opposed to alkaloids, glycosides, Phenols and Tannins. Extracts from Kitale recorded high presence of alkaloids, steroids and flavonoids, as opposed to glycosides, saponins, terpenoids phenols and tannins. Extracts from Kericho recorded high presence of alkaloids, saponins, steroids and flavonoids, as opposed to glycosides, terpenoids phenols and tannins. Finally, extracts from Marigat recorded high presence of saponins, terpenoids, steroids and tannins, as opposed to alkaloids, glycosides, phenols and flavonoids. It is worth noting that *U. massaica* from all the regions exhibited high levels of steroids as indicated in Table 3.

Table 3: Phytochemical analysis of *U. massaica* crude extracts

Phytochemical Screening	Eldoret	Kitale	Kericho	Marigat
Alkaloids	+	++	++	+
Glycosides	+	+	+	+
Saponins	++	+	++	++
Terpenoids	++	+	+	++
Phenols	+	+	+	+
Steroids	++	++	++	++
Tannins	+	+	+	++
Flavonoids	++	++	++	+

(++) Conspicuously Present (+) Discreetly Present

Discussion

Results indicate that inhibition zones among the solvents and/or control varied significantly (P<0.05).

These results further showed that the methanolic crude extracts showed higher inhibition zones than the aqueous extract. This could be attributed to different polarity and extracting potential of methanol and water. Methanol can dissolve both polar and non-polar substances. A study reported that most antimicrobial agents that have been identified from plants are soluble in organic solvents and this reveals the better efficiency of ethanol as extracting solvent than water [25].

Similar findings were reported where high antibacterial activities observed in the methanolic extracts than aqueous extract of selected medicinal plants [26]. Findings from the present study further indicated that aqueous *U. massaica* extract did not inhibit the growth of *E. coli.* these findings are contrary to those carried out on plants with a medicinal value such as *Allium sativum* which shown antimicrobial activity against *Escherichia coli*. The variation could be attributed to the part of the part and moreover, the plants used could be of the different age [27]. Generally, methanolic crude extract of *U. massaica* recorded antimicrobial activity against the test organisms; *S. aureus, E. coli, A. flavus* and *C. albicans.* The findings were similar to those obtained in a study carried out on Gram negative and Gram positive bacteria as well as fungi in India and England; on *Escherichia coli* and on *Staphylococcus aureus* in Nigeria; found out that, extracts from a wide range of medicinal plants including *U. massaica* had antimicrobial activity against the test microorganisms [28, 29, 30]. Moreover, findings from this study are in consonance with findings of other researchers who reported antimicrobial activity in methanolic extracts of *Aloe vera* against *S. aureus* (18 mm) and *E. coli* (8.46 mm) at a using agar-well diffusion method [31].

The antimicrobial activities of *U. massaica* extracts could be due to the presence of bioactive ingredients. In addition, active ingredients not to be significant such as phenols have been reported to confer broad spectrum antibacterial activities [31]. These results, together with ethnobotanical studies made previously by other investigations, suggest that *U. massaica* might have important compounds that can potentially be used for to inhibit pathogenic microorganisms [32, 33].

Conclusion

Generally, the crude *U. massaica* extracts showed antimicrobial activity when used against *S. aureus* and *E.coli* Higher inhibition zone range of 6.33mm to 8.4mm was recorded in methanolic crude extract compared to 6-6.67mm of aqueous crude extract of the plant, we concluded that methanol is a better solvent than water. There is need to do further cytotoxicity test.

Acknowledgements

The authors wish to thank the Kenya Medical Research Institute (KEMRI) for providing the facilities and what it took to accomplish the research.

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