

Original Research Article

Phytochemical Constituents and Anti-Salmonella Activity of the Combined Leaves Extracts of Selected Medicinal in Mubi, Nigeria

ABSTRACT

This study was aimed at determining the quantitative phytochemical constituents and anti-salmonella effect of the combined leaves ethanolic and aqueous extracts of selected plants in Mubi. The leaves of *Citrus sinensis*, *Senna siamea*, *Moringa oleifera* and *Carica papaya* were collected and shade dried; and consequently grounded and mixed in equal ratio. The combined powdered plants leaves was extracted using ethanol and water. The *Salmonella typhi* and *Salmonella paratyphi* used were obtained from the laboratory section of General Hospital, Mubi. The results of phytochemical analysis showed that the ethanolic extract had the significantly highest content of compounds like: tannins (4.96 mg/100g), alkaloids (8.45 mg/100g), flavonoids (3.00 mg/100g), saponins (9.12 mg/100g) and phenols (26.10 mg/100g), while the aqueous extract had lowest of all the compounds. The anti-salmonella activity test showed that the highest concentrations (200 mg/ml) of the ethanolic and aqueous extracts recorded the highest zone of inhibition of 0.73 and 0.70 cm and 0.60 and 0.80 cm on the *S. typhi* and *S. paratyphi*, respectively; while the lowest was recorded by the lowest concentrations of the extracts. However, the control used (ceftriaxone) was more effective against the two test organisms than the highest concentrations of the two extracts. The study concluded that the ethanolic extract of the combined leaves of *S. siamea*, *C. papaya*, *C. sinensis* and *M. oleifera* has higher phytocompounds than the aqueous extract; the anti-salmonella activity of ethanolic and aqueous extracts on *S. typhi* have no significant difference, while on *S. paratyphi*, aqueous extract is more effective than ethanolic extract.

Keywords: Anti-bacterial, Efficacy, Phytochemical, Quantitative analysis

1. NTRODUCTION

The use of plants for medicinal purposes is as old as mankind. The numerous plants which proved to be medicinal are used by traditional medicine practitioners as a remedies to some diseases which include: diabetes, sweating, bleeding, regulation of menstrual cycle, stomach pain, inflammation and toothache [1,2]. Almost 80 % of human population worldwide, majority of which are in African and other developing countries, still depends mainly on these medicinal plants in the treatment of different diseases [3]. Most of these medicinal plants have shown to have no adverse or toxic effect on humans that used them while some, however, have toxic effect on both humans and animals [3].

Plants used for medicinal purposes contained some active compounds or raw materials which are being used in the development of some modern drugs or for extracting essential oils [4]. *C. sinensis* which was reported to have antimicrobial, anti-diabetic and antiviral effect; and used as insect repellent [5] is an important source of vitamin C and phytocompounds like phenolics and carotenoids known to be of great benefit in maintaining good health [6]. Extracts from *C. papaya* fruit skin, pulp and seeds and the leaves exhibited an antibacterial effect against some bacteria

like: *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhi* [7]; and used in the treatment of helminthiasis [8]. Phytochemical analysis of the leaves indicated that it contain saponins, cardiac glycosides and alkaloids [9]. The leaves of *S. siamea* are used medicinally as anti-malaria, laxative, blood cleaning agent, cure for digestive system disorder, herpes, swine fever, syphilis, typhoid and lot of other diseases [10,11]. The leaves extract of the plant was reported to contain alkaloids, flavonoids, glycosides, saponins, tannins, terpenoids, phenols and steroids; and had an antibacterial effect against *S. typhi*, *Shigella* spp, *E. coli* and *Pseudomonas aeruginosa* [12]. The leaves extracts of *M. oleifera* was also proved to have antibacterial effect; and contained some bioactive compounds such as flavonoids, saponins, alkaloids, tannins and steroids [13]. The leaves or roots of the plant has been in used by the traditional medicine practitioners in the treatment of skin infections, eye and ear infections, pain in joints, pimples, sore throat, respiratory disorder, headaches and many other diseases [14].

Salmonella is a genus of Gram-negative, flagellated anaerobic bacilli bacteria that is characterized by O, H and Vi antigens [15]. *Salmonella typhi* is a member of this genus that is responsible for typhoid fever disease. This disease is very common in developing countries where access to safe water supply and adequate sewage disposal are lacking [16]. The use of antibiotics in the treatment of diseases associated with *Salmonella* species have not been very successful due to resistance developed by the different species of bacteria from this genus. Drug resistance strains of this *Salmonella* species are now found globally in both developed and developing countries [17]. A highly virulent *Salmonella*, that are resistant to virtually all available antibiotics, thus causing high rate of death in humans have been witnessed since the last few decades [18]. Due to the antibiotic-resistance developed by *Salmonella* and their high cost, most people in Africa especially in Nigeria, have resorted to the use of extracts from medicinal plants like: *M. oleifera*, *C. sinensis*, *S. siamea* and *C. papaya*. An extracts from the blend of different parts of these plants are mostly used by people in the treatment of typhoid fever. Therefore, in order to scientifically authenticate the effectiveness of extracts from the blend of these plants parts against *S. typhi* and *S. paratyphi*, **in-vitro** test and phytochemical constituents of these plants extracts were deemed necessary in this study.

2.0 MATERIALS AND METHODS

2.1 Preparation of Plant Samples and Study Area

The leaves samples of *C. sinensis*, *C. papaya*, *M. oleifera* and *S. siamea*, which were collected from within Mubi town were shade dried after being authenticated by a taxonomist in the Department of Botany, Adamawa State University, Mubi; and later grounded separately into fine powder. The powder leaves samples were mixed in the ratio of 25:25:25:25 so as to get 100 g of the four (4) plants powdered leaves samples.

2.2 Extraction of Plant Materials

Maceration method of extraction as described by [19], using water and ethanol as solvents was used for the extraction of the plants bioactive components. The solvents from the extract were removed using evaporating dish placed on water bath set at 50°C. Finally, the residue was then collected and kept in a refrigerator at 4°C in a specimen bottle pending analysis.

2.3 Quantitative Analysis of Phytochemical Constituents

2.3.1 Determination of total phenols

Analysis of the total phenols was carried out according to the method described by [20]. A fat free sample was boiled with 50 ml of petroleum ether for the extraction of the phenolic component for 15 minutes. The extract (5 ml) was then pipetted into a 50 ml flask, and 10 ml of distilled water was added. Ammonium hydroxide solution (2 ml) and 5 ml of concentrated amylalcohol were added also. The sample was made up to mark with distilled water and left to react for about 30 minutes for colour development. This was measured at 505 nm using spectrophotometer.

2.3.2 Determination of tannins

The method of [21] was used in analyzing the tannins content of the plant materials. A measured weight of the sample (1 g) was dispersed in 10 ml distilled water and agitated for 30 minutes at room temperature being shaken every 5 minutes. At the end of the 30 minutes, it was centrifuged and the extract gotten. Then 2.5 ml of standard tannic acid solution was dispersed into a separate 50 ml flask. A 1 ml folin Denis reagent (which is a mixture of phosphomolybdate and phosphotungstate) was measured into a flask followed by 2.5 ml of saturated sodium trioxocarbonate (Na_2CO_3) solution. The mixture was diluted to mark with distilled water in the flask (50 ml) and incubated for 90 minutes at room temperature. The absorbance was measured at 725 nm using spectrophotometer. The tannin content was calculated as follows:

$$\% \text{ tannin} = A_n / AS \times C \times 100 / W \times V_f / V_a$$

Where: A_n = Absorbance of test sample

AS = Absorbance of standard solution

C = Concentration of standard solution

W = Weight of sample used

V_f = Total volume of extract

V_a = Initial volume of extract

2.3.3 Determination of flavonoids

The flavonoids content was determined using the method described by [22]. About 10 g of the plants sample was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was then 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.

2.3.4 Determination of saponins

The method of [23] was used in determining the saponins content.

2.3.5 Determination of alkaloids

About 5g of the sample was weighed into a 250ml beaker and 80ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours. This was filtered and the extract was

concentrated on a water bath to one quarter of the original volume. The whole solution was allowed to settle and the precipitate was collected and washed with diluted ammonium hydroxide and then filtered. The residue is the alkaloids, which was dried and weighed.

2.4 Antibacterial Activity Testing

2.4.1 Source of bacterial isolates

The bacterial isolates which include *Salmonella typhi* and *Salmonella paratyphi* were clinical isolates obtained from the laboratory section of General Hospital, Mubi.

2.4.2 Preparation of different concentrations of the plant extract

The plants extracts were prepared into four (4) different concentrations (i.e 25, 50, 100 and 200 mg/ml). The extract concentrations were prepared by weighing 2 g of the extract into 10 ml of sterile distilled water (200 mg/ml). A doubling dilution of the diluted extract was carried out into three (3) different labeled bottles to obtain concentrations 100, 150 and 50 mg/ml respectively.

2.4.3 Standardization of the inocula

The test organisms (inocula) were prepared by streaking the organisms on the freshly prepared nutrient agar plates to obtain discrete bacterial colonies. A colony was then picked and sub cultured **onto** sterile nutrient broth and incubated at 37 °C for 24 hours. After the incubation period, a loopful of broth culture was transferred into a bottle containing sterile distilled water so as to obtain a bacterial cell density of 1.5×10^8 cfu/ml as determined by McFarland turbidity standard (Scale number one).

2.4.4 Susceptibility testing of the extracts

This was carried out using Agar well diffusion method. The standardized organisms were uniformly streaked **onto** freshly prepared Mueller Hinton Agar with the aid of a sterile swab stick (cotton swabs). Four wells were punched on the inoculated agar plates using a sterile cork borer of 6 mm and were properly labeled. The punched wells were then filled with 0.2 ml of each the extracts. **Ceftriaxone 500 mg was used as a positive control.** The plates were allowed to stay on the bench for 1 hour for the extract to diffuse into the agar and were later incubated at 37°C for 24 hours. After the incubation period, the plates were observed for any evidence of inhibition, which appeared as clear zones that were completely devoid of growth around the wells. The diameter of the clear zones was measured with a transparent ruler calibrated in centimeter (cm).

3. RESULTS AND DISCUSSION

The quantitative phytochemical analysis of the ethanolic and aqueous extracts of combined leaves of *S. siamea*, *C. papaya*, *C. sinensis* and *M. oleifera* showed that the ethanolic extract had the statistically significantly highest (at $p \leq 0.05$) contents of phytochemical constituents which include: tannins (4.96 mg/100g), alkaloids (8.45 mg/100g), flavonoids (3.00 mg/100g), saponins (9.12 mg/100g) and phenols (26.10 mg/100g) than the aqueous extract which had the significantly lowest of these compounds (Table 1). Quantitative phytochemical analysis of *M. oleifera* ethanolic and aqueous leaf extracts showed lower content of phytochemicals **like total phenols, saponins and alkaloids** than the one analyzed in this study [24,25]. Similarly, the phytochemical contents of *C. papaya* ethanolic leaf extract as reported by [26] was observed to be lower than that of the ethanolic extract of this study. The higher content of the phytochemical components of the ethanolic and aqueous extracts analyzed in this study than that of the individual plant could be attributed to the fact that the two extracts were a pull of the leaves of four (4) plants each of which had contributed to the individual phytochemical constituents. As shown in table 1 also, the ethanolic extract had the higher contents of all the phytochemical

components analyzed than the aqueous extract. This could be as a result of the ethanol having a better capacity to dissolve most phytochemicals than water as similarly reported by [27].

The anti-bacterial activity test of the ethanolic and aqueous combined leaves extracts of *S. siamea*, *C. papaya*, *C. sinensis* and *M. oleifera* showed that the highest concentration of ethanolic extract (200 mg/ml) recorded the second highest zone of inhibition on both *S. typhi* and *S. paratyphi* after the control (ceftriaxone) with 0.73 and 0.70 cm respectively and the lowest zone of inhibition (0.10 and 0.20 cm respectively) was that of the concentration 25 mg/ml which was significantly similar to that of concentration 50 mg/ml. Similar event was observed for the aqueous extract on *S. typhi* and *S. paratyphi* as the extract had the second highest zone of inhibition after the control which had the statistically significantly highest zone of inhibition (Table 2). The antibacterial activity exhibited by the combined leaves extracts against *Salmonella* species is indeed not a surprise as each of the individual plant leaves that constitute the two extracts all showed anti-salmonella activity with extracts of organic solvents having the highest inhibitory effect than aqueous extracts [28,29,30,31]. The results of this study agrees with the findings of [27] who reported an inhibitory effect of the combined leaves extracts of *C. siamea*, *Coffea senna* and *Citrus lemon* against some human pathogenic bacterial species involving *S. typhi*, *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pneumoniae*. Similarly, the combined leaves extracts of some medicinal plants which include: *Bryophyllum pinnatum*, *Ocimum gratissimum*, *Jatropha curcas* and *Ficus exasperata* was reported to be effective against *S. typhi* and some other enteric bacterial species [32].

Comparison of the effect of the ethanolic and aqueous extracts on the two test organisms as indicated in table 3 showed that there is no statistical significant difference in the effect of the two extracts on *S. typhi*, but on *S. paratyphi* with the aqueous extract having the significantly highest inhibitory effect. The study of [27] similarly discovered the effectiveness of aqueous extract over that of an organic solvent (n-hexane) on virtually all the bacteria species on which the extracts were tested. Although, the ethanolic extract had higher effect on *S. typhi* than the aqueous extract, but statistically there is no significant difference. The high inhibitory effect of the ethanolic extract on *S. typhi* might not be too far from that fact that alcoholic extracts contain more dissolved antimicrobial properties effective against the test organism as shown in table 1 than aqueous [33,34].

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the ethanolic and aqueous extracts of the combined leaves of *S. siamea*, *C. papaya*, *C. sinensis* and *M. oleifera* showed that the ethanolic extract had concentration 3.13 mg/ml as MIC on both *S. typhi* and *S. paratyphi* and 6.25 and 3.13 mg/ml as MBC on *S. typhi* and *S. paratyphi* respectively (Table 4). The MIC of *M. oleifera* and *Gongronema latifolium* extracts mixture against *S. typhi* was reported to be between 12.5-25.0 mg/ml, while the MBC was 6.25-12.5 mg/ml [35]. The lower MIC reported in this study in comparison with that of [35] might be attributed to the extracts being a mixture of four (4) medicinal plants that were reported to have an inhibitory effect against *Salmonella* species and many other bacterial species [28,29,30,31].

Table 1: The Quantitative Phytochemical Constituents of the Ethanolic and Aqueous Combined Leaves Extracts of *S. siamea*, *C. papaya*, *C. sinensis* and *M. oleifera*

Extract	Phytochemical (mg/100g)				
	Tannins	Alkaloids	Flavonoids	Saponins	Phenols
Ethanolic	4.96±0.09 ^a	8.45±0.07 ^a	3.00±0.08 ^a	9.12±0.07 ^a	26.10±0.11 ^a
Aqueous	2.67±0.08 ^b	3.12±0.07 ^b	2.05±0.07 ^b	6.78±0.06 ^b	17.23±0.07 ^b

p-Value	0.00	0.00	0.00	0.00	0.00
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Means along the column with different superscript alphabet are statistically significantly different at $p \leq 0.05$.

Table 2: The Antibacterial Activity of the Ethanolic and Aqueous Combined Leaves Extracts of *S. siamea*, *C. papaya*, *C. sinensis* and *M. oleifera*

Concentration (mg/ml)	Zone of Inhibition (cm)/Extract			
	Ethanolic		Aqueous	
	<i>S. typhi</i>	<i>S. paratyphi</i>	<i>S. typhi</i>	<i>S. paratyphi</i>
25	0.10±0.02 ^d	0.20±0.03 ^d	0.10±0.02 ^d	0.40±0.09 ^c
50	0.20±0.03 ^d	0.40±0.08 ^{cd}	0.20±0.04 ^{cd}	0.60±0.08 ^{bc}
100	0.50±0.04 ^c	0.60±0.09 ^{bc}	0.40±0.09 ^{bc}	0.70±0.08 ^b
200	0.73±0.08 ^b	0.70±0.10 ^b	0.60±0.08 ^b	0.80±0.11 ^b
Ceftriaxone (500 mg)	1.20±0.06 ^a	1.30±0.10 ^a	1.20±0.06 ^a	1.30±0.09 ^a
p-Value	0.00	0.00	0.00	0.00

Means along the column with the same superscript alphabet are not statistically significantly different at $p \leq 0.05$.

Table 3: Comparison of the Efficacy of the Ethanolic and Aqueous Combined Leaves Extracts of *S. siamea*, *C. papaya*, *C. sinensis* and *M. oleifera* against *S. typhi* and *S. paratyphi*

Treatment	Zone of Inhibition (cm)/Test Organism	
	<i>S. typhi</i>	<i>S. paratyphi</i>
Ethanolic	0.55	0.64
Aqueous	0.50	0.76
SE±	0.03	0.03
p-Value	0.22	0.04

Means along the column with the same superscript alphabet are not statistically significantly different at $p \leq 0.05$.

Table 4: The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Ethanolic and Aqueous Combined Leaves Extracts of *S. siamea*, *C. papaya*, *C. sinensis* and *M. oleifera* on *S. typhi* and *S. paratyphi*

EXTRACT	MIC (mg/ml)		MBC (mg/ml)	
	<i>S. typhi</i>	<i>S. paratyphi</i>	<i>S. typhi</i>	<i>S. paratyphi</i>
Ethanolic	3.13	3.13	3.13	3.13
Aqueous	6.25	3.13	6.25	3.13

4. CONCLUSION

The combined leaves ethanolic extract of *M. oleifera*, *S. siamea*, *C. sinensis* and *C. papaya* contain phytochemical constituents which include: tannins (4.96 mg/100g), alkaloids (8.45 mg/100g), flavonoids (3.00 mg/100g), saponins (9.12 mg/100g) and total phenols (26.10 mg/100g) that are significantly higher than of the aqueous extract which has tannins (2.67 mg/100g), alkaloids (3.12 mg/100g), flavonoids (2.05 mg/100g), saponins (6.78 mg/100g) and total phenols (17.23 mg/100g).

The ethanolic and aqueous extracts of the combined leaves of *M. oleifera*, *S. siamea*, *C. sinensis* and *C. papaya* both have an anti-salmonella effect against *S. typhi* and *S. paratyphi*. Therefore, the use of extracts from the combined leaves of *M. oleifera*, *S. siamea*, *C. sinensis* and *C. papaya* in the treatment of typhoid fever and some other diseases of *Salmonella* species by most people in Nigeria is justifiable.

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