Original Research Article

The Phytochemical Constituents and Anti-Salmonella Activity of the Combined Leaves Extracts of Selected Medicinal Plants

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. 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 (ceftriazone) 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.

Comment [MOU1]: ceftriaxone

Keywords: Anti-bacterial activity test, Efficacy of combined leaves extract, Phytochemical constituents, Quantitative analysis

1.0 INTRODUCTION

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. 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 subtillis* 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*, invitro 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

The collected leaves samples of *C. sinensis*, *C. papaya*, *M. oleifera* and *S. siamea* were shade dried; and later grounded separately into fine powder using wooden pestle and mortar. 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.

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The authors must include who authenticated the species

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.

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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].

2.3.2 Determination of tannins

The method of [21] was used in analyzing the tannins content of the plant materials.

2.3.3 Determination of flavonoids

The flavonoids content was determined using the method described by [22].

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 unto sterile nutrient broth and incubated at 37 ^{0}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 unto 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. 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^{0}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).

3.0 RESULTS AND DISCUSSION

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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 phytocompounds 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 phytocompounds than water as similarly reported by [27].

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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 (ceftriax~~z~~one) 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*, *Coffee senna* and *Citrus lemon* against some human pathogenic bacterial species involving *S. typhi*, *Staphylococus aureus*, *Escherichia coli* and *Streptococcus pneumonia*~~)~~.

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 [32,33].

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

	Phytocompound (mg/100g)				
Extract	**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

Means along the column with different superscript alphabet are statistically significantly different at p≤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*

	Zone of Inhibition (cm)/Extract			
	Ethanolic		**Aqueous**	
Concentration (mg/ml)	***S. typhi***	***S. paratyphi***	***S. typhi***	***S. paratyphi***
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	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≤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*

	Zone of Inhibition (cm)/Test Organism	
Treatment	***S. typhi***	***S. paratyphi***
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≤0.05.

4.0 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.

REFERENCES

1. Selim S, Adam ME, Hassan SM, Albalawi A. Chemical composition, antimicrobial and antibiofilm activity of the essential oil and methanol extract of the Mediterranean cypress (*Cupresses sempervirens* L). BMC Complement Avern Medicine. 2014;14(179):14-16.
2. Shafaghat A. Antibacterial activity and composition of essential oils from flower, leaf and stem of *Chaerophyllum macropodum* Boiss. from Iran. Natural Product Communication. 2009; 4(6):749-888.
3. Okoye TC, Uzor PF, Okereke EK. Safe African medicinal plants for clinical studies. In: Victor, K. (Ed.) Toxicological survey of African medicinal plants. 2014; 535-555.
4. Jugreet BS, Mahomoodally M. Pharmacological properties of essential oil constituents and their mechanisms of action. In: Swamy, M. (Ed.) plant –derived bioactives, Springer, Singapore; 2020.
5. Fujikawa T, Iwanami T. Sensitive and robust detection of citrus greening (huanglongbing) bacterium *Candidatus Liberibacter asiaticus* by DNA amplification with new 16S rDNA specific primers, Molecular and Cellular Probes. 2012;26:194-197.
6. Martinez-Cuenca MR, Primo-Capela A, Forner-Giner MA. Influence of rootstock on Citrus tree growth effects on photosynthesis and carbohydrate distribution, plant size, yield fruit quality and dwarfing genotype. In-Tech; 2016.
7. Aravind G, Bhowmik D, Duraivel S, Harish G. Traditional and medicinal uses of *Carica papaya*. Journal of Medicinal Plants Studies. 2013;1(1), 7-15.
8. Anuar NS, Zahari SS, Taib IA, Rahman MT. Effect of green and ripe *Carica papaya* epicarp extracts on wound healing and during pregnancy. Food and Chemical Toxicology. 2008; 46(7), 2384-2389.
9. Okwu DE. Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. Journal for Sustainable Agriculture Environment. 2004; 6(1), 30-37.
10. Abdulkadir AG, Ibrahim H, Kendeson AC, Abdullahi Y. Comparative Analysis of PhysicoChemical Parameters of Borehole, Pond and Well Water in Kashere Metropolis. European Journal of Engineering Research and Science. 2017;2(6):42-27.
11. Abdul Z, Dimas K, Sunday AO, Isyaka MS, Said JA. Quantitative Investigation of phytochemicals and brine shrimp lethality test of the root, stem bark and leave extract of *Isoberlia doka*. International Journal of Chemical Studies. 2015;3(3):36-38.
12. Nas FS, Oyeyi TI, Ali M. Antibacterial efficacy and phytochemical screening of *Senna siamea* leaves extracts on some pathogenic bacteria. Journal of Microbiology and Experimentation. 2018; 6(3):159-163.
13. Amabye TG, Tadesse FM. Phytochemical and antibacterial activity of *Moringa oleifera* available in the market of Mekelle. Journal of Analytical and Pharmaceutical Research. 2016; 2(1):23-26.
14. Anwar F, Latif S, Ashraf M, Cutani AH. *Moringa Oleifera*: A Food plant with multiple medicine uses. Phytotherapy Research. 2007;21:17-25.
15. Giannella RA. Salmonella. 4th edition, University of Texas Medical Branch, Galveston; 1996.
16. Buckle GC, Walker CL, Black RE. Typhoid fever and paratyphoid fever: Systematic review to estimate global morbidity and mortality for 2010. Journal of Global Health. 2012; 2(1), 10398-10401.

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17. Threlfall EJ. Antimicrobial drug resistance in *Salmonella*: problems and perspective in food and water-borne infectious. FEMS Microbiology Revolution. 2002;26(2):141-148.
18. Nair DVT, Venkitanarayanan K, Johny AK. Antibiotic-resistant *Salmonella* in the food supply and the potential role of antibiotic alternatives for control. Foods. 2018;7:167.
19. Nagappan R. Evaluation of aqueous and ethanol extract of bioactive medicinal plant, *Senna didymobotrya* (Fresenius) Irwin and Barneby against immature stages of Filarial vector, *Culex quinquefasciatus* Say (diptera:Culicidae). Asian Pacific Journal of Tropical Biomedicine. 2012;2(9):707-711.
20. Padamanabhan V, Manimekalai G, Vasthi KE, Nirmala A, Jagajothi A. Phytochemical screening and antioxidant activity of extracts of the leaf and bark of *Albizzia lebbeck* (Benth). Academia Journal of Medicinal Plants. 2014; 2(2):026-031.
21. Pearson D. Chemical analysis of food. Churchill Livington, Edinburgh, UK. 1976;103-110.
22. Boham BA, Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of *Hwaiian vaccinium vaticulatum* and *V. calycinium*. Pacific Science. 1994;48:458-463.
23. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Science. 2001; 8:203-208.
24. Ajantha A, Kathirvelan C, Purushothaman MR, Visha P. Studies on qualitative and quantitative phytochemical constituents of *Moringa oleifera* leaf meal. International Journal of Current Microbiology and Applied Sciences. 2020;9(6):4195-4201.
25. Nweze NO, Nwafor FI. Phytochemical, proximate and mineral compositions of leaf extracts of *Moringa oleifera* Lam. from Nsukka, South Eastern Nigeria. Journal of Pharmacy and Biological Sciences. 2014; 9(1):99-103.
26. Omidiwura BRO. Qualitative and quantitative analysis of pawpaw (*Carica papaya*) leaf extract and its antimicrobial effect in animal production. Nigerian Journal of Animal Production. 2017; 44(3):78-83.
27. Abduallhi Z, Danja BA, Abdulaziz HG, Jibrin S, Abubakar A, Abdu Z, Hammashi LH, Umar M, Ahmad AT, Said SS. Phytochemicals properties and antibacterial activity of combined leaves extract of *Senna siamea, Coffee senna* and *Citrus lemon*. International Journal of Scientific and Engineering Research. 2019; 10(3):1049-1057.
28. Enerijiofi KE, Akapo FH, Erhabor JO. GC-MS analysis and antibacterial activities of *Moringa oleifera* leaf extracts on selected clinical bacterial isolates. Bulletin of the national research centre. 2021;45:179.
29. Mohammed N, Sanyinna YM, Ahmad RN. Antibacterial and antifungal activities of *Citrus sinensis* leaves extracts. International Journal of Scientific Research in Biological Sciences. 2021; 8(6):01-08.
30. Nirosha N, Mangalanayaki R. Antibacterial activity of leaves and stem extract of *Carica papaya* L. International Journal of Advances in Pharmacy, Biology and Chemistry. 2013; 2(3):473-476.
31. Doughari JH, Okafor NB. Antibacterial activity of *Senna siamea* leaf extracts on *Salmonella typhi*. African Journal of Microbiology Research. 2008; 2(1):042-046.
32. Parekh J, Jadeja D, Chamada S. Efficiency of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turkish Journal of Biology. 2005; 29:203-210.

33. Voravuthikunchai S, Lortheeanuwat A, Jeeju W, Sririrakphongpaichit S, Supawita T. Effective medicinal plant against enterohaemorrhagic *Escherichia coli*. Journal of Ethanopharmacology. 2004;94:49-54.