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EFFICACY AND PHYTOCHEMICAL PROFILES OF LEAF EXTRACT OF YELLOW TASSEL (EMILIA SONCHIFOLIA) PLANT ON SELECTED DIARRHOEAGENIC PATHOGENS

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ABSTRACT

Emilia sonchifolia (Composite) has found various medicinal uses in folk medicine, as a cure for various ailments such as sore throat, wound healings, stomach ache, conjunctivitis, depurative, anticonvulsants, bowel complaint remedies and sores, aqueous extract is used to treat internal heat among pregnant women. The need to pharmacologically confirm these claims stimulated the need for this investigation. The effect of aqueous extract of Emilia sonchifolia on some diarrhoeagenic pathogens like Escherichia coli, Shigella spp. Salmonella, Pseudomonas, Aeruginosa, and Staphylococcus aureus were investigated. The Emilia sonchifolia leaves were soaked for 48hrs (2 days) and the undiluted and diluted extracts used to inoculate five agar plates containing the pathogens. 0.1ml of the diluted and undiluted extracts was administered to the organisms to show various sensitivity and resistance percentages. In the undiluted extract of Emilia sonchifolia, E.coli and Staphylococcus aureus pathogens shows a high sensitivity of over 80% each with about 70% inhibition (R). Shigella species on the other hand showed 60% sensitivity with about 40% inhibition (R). Salmonella species and Pseudomonas aeruginosa each showed 40% sensitivity and 20% inhibition (R). In the diluted extract of Emilia sonchifolia on the isolates, E. Coli showed 60% sensitivity (s) with and 20% inhibition (R), Shigella species and Pseudomonas aeruginosa each shows 40% sensitivity against the diluted extract and 20% inhibition (R), while Staphyllococcus aureus showed 20% sensitivity(s) 40% and 20% inhibition (R). Salmonella spp showed 20% sensitivity (s) and 10% inhibition. Percent zone of growth inhibition of pathogens was higher with undiluted extracts compared to diluted extracts. Results of phytochemical screening of the leave extracts of Emilia sonchifolia showed that the plant leaves extract contains in order of high concentrations and efficacy of phytochemicals; tannins > triterpenoids = saponins = anthraquinones = flavonoids > alkaloids = reducing sugars. There was no steroid compound found in the extract suggesting that the plant is steroid free and ideal for human medicinal formulations.

KEYWORDS: Diarrhoeagenic pathogens, Aqueous leaf extracts, Yellow Tassel Flower, sensitivity, zone of inhibition.

INTRODUCTION

Over the years, man has used plant (whole or parts) for food, aesthetic beautification, and medicine and for the feeding of livestock. Plants have economic values and are not only used for mere satisfaction or sustenance of hunger but also for the maintenance of good health (Alter, et al., 2003).

Plants are rich sources of natural products, they form the major parts of ingredient in almost all system of therapeutics pharmaceutical industries are conducting extensive research on plants collected from the rain forest and other places for their potential medicinal values; modern allopathic system of medicine is also based on plants and herbs. Medicinal plants are relevant in both developing and developed nations of the world as sources of drugs or herbal extracts for the various chemotherapeutic purposes continue to play a dominant role in maintaining human health since antiquities (Comfort and Ogbonnaya, 2000).

Over 50% of all modern clinical drugs are of natural product origin and natural products plays an important role in drug development programme of the pharmaceutical industry. In the continuation of this strategy of new drug discovery, emphasis has been laid on the aerial parts of most plants for their antibacterial and anti-oxidant properties. Gayanthri (2012) had posited that modern clinical drugs of natural products plays an important role in pharmaceutical industries, chemicals which are naturally present in plants are converted

traditionally and medicinally into substances that regulate human fertility. Some secondary compounds produced by plants could be very effective against parasites and pathogens such as plants include pawpaw, mango, citrus as well as Emilia sonchifolia (Brianna et al., 2011). Emilia sonchifolia (composite) is an herbaceous plant which grows up to about 10 – 40cm in height. Develops ripe fruits between August to October, the flowers are hermaphrodite and are pollinated; the plant is pantropic and probably originated from South Asia (Hasegawa, 2000).

Emilia sonchifolia has been found to be very effective in the treatment of several diseases, such as sore throat, wound healings, conjunctivitis, infantile diarrhoea and the like. In India for instance, the leaves extract of this plant have been used to treat dysentery (Arora and Arora, 2008). The aerial part is believed to contain flavonoids, terpenes and alkaloids. In Africa, the tea made from the leaves of Emilia sonchifolia is used in folk medicine for the treatment of dysentery. The juice extract of the leaves is used for the treatment of cuts and wound, sore ears etc. The juice of the root is used for the treatment of diarrhoea while the flower heads are kept in the mouth for some minutes to prevent tooth decay (Essien et al., 2009).

In Cameroun, it is used as a local remedy for craw – craw and in Brazil, the aqueous extract of the leaves and whole plants have been used to treat flu, cold and fever, diarrhoea, rheumatism and spasm (Couto et al., 2000). The leaves have also been exploited as tea plant for the anti-inflammatory, analgesic, anti-diarrhoeal agent and for the healing of gynaecological diseases. It is used as emetic, stomach ache dysentery and bowl complaints (Ekukudo, 2001).

Duke and Ayensu (2005) had also reported that the juice of the leaves is used in the treatment of eye inflammation, night blindness, cuts and wounds as well as ear infections. The plant leaves extract have also been reported to be an astringent depurative used for the treatment of infantile lympanities and bowl constipations.

In central Africa, Emilia sonchifolia has been widely used in the treatment of pneumonia, wound healing and burns, skin diseases, mental retardation, infectious diseases, migraine headache, asthma, abdominal heat reduction in pregnant women and urinary infections (Mcfarl and Lynn, 2007).

Apart from its medicinal value, Emilia sonchifolia have high nutritional value as an edible plant where is serves as a mild laxative agent. Phytochemical screening of the plant by Muko and Ohiri, (2000) have revealed that the plant contains palmitic acid, alkaloids 0.2%, aechuain (isoflavone), doronine, kuemferol, rhamnopyranoside, 1-2, B-D –glycopyranoside, meanrsetin, simiral and senkinine some of which are very effective against diarrhoeagenic pathogens.

Diarrhoea is caused by various bacterial pathogens like Escherichia coli, Vibrio parahemolytica, Shigella spp. In different disease conditions such as cholera, dysentery and diarrhoea, Diarrhoea is a condition of passing out frequent water stool as sign of cholera, dysentery and other diseases. There is a high rate of diarrhoea in the tropics especially in fruiting seasons (Cheng and Redder, 2004). Diarrhoea pathogens inhabit the guts of sufferers as parasite through poor food hygienic conditions, poor sanitation and HIV/AIDs. Diarrhoea affects more than 2.2 million people Worldwide annually according to WHO reports of 2003. Diarrhoea leads to rapid dehydration of body fluids among sufferers and may be fatal if body fluids are not immediately replaced. Thus, it is against this backdrop, that this paper seeks to evaluate the potency of leaves extracts of Emilia sonchifolia against diarrhoeagenic pathogens in Nigeria.

MATERIALS AND METHODS

Plant materials

Plant materials used for the study were the leaves of Emilia sonchifolia obtained from the botanical garden of the University of Calabar, Calabar Nigeria.

Preparations of plant extracts

Fresh leaves of Emilia sonchifolia plants were carefully harvested and washed in distilled water to remove dirts and other contaminants. The leaves were then chopped into smaller pieces to allow for easy drying. The chopped leaves were air dried for eight days at room temperature. Leaves were not sun dried to avoid loss of volatile compounds through ultraviolent radiation. The air dried leaves were carefully grinded into powdered form using manual miller.

Forty (40g) of powdered leaves was weighed and soaked in beaker containing 400ml of distilled water for 48 hours (2days). The resultant mixture was filtered using cheese cloth and sieve to get the extract. The filtrate was centrifuged at 3000rpm for 5 min. after which the supernatant was carefully discarded using pipette and leaving the residue which was a blackish- green paste. This was collected and preserved in a refrigerator at for future usage.

Preparation of extract concentrations

The solvent was then poured out into centrifuge tube and spin at 1000rpm for 10 min. from the resulting stock solution. 100ml was separated and was our 100% concentration of leaf extract. The remaining solution was serially diluted to obtain the different concentrations of 0%, 10%, 20%, 30%, and 40% respectively.

Diarrhoeagenic pathogens isolates used for the study

Five (5) bacterial pathogens associated with human diarrhoea symptoms were classified and used for the study and they included Shigella spp., Salmonella spp.,

Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus.

Culturing of diarrhoeagenic pathogens isolates and application of leaf extracts

The disc diffusion techniques of Kirby-Bauer was adopted as modified by Stakes and Ridway, (2001) in testing for the potency and efficacy of the leave extracts for antimicrobial sensitivity of the known cultures.

The sensitivity disc used for sensitivity testing was punched from one end and a Whitman's filter paper was used to place on the punched side. Sterile disc of 5mm diameter was loaded with the raw leaf extracts of Emilia sonchifolia, while some others were loaded with dilutions of the extracts and dried in an oven at 60°C for 5 min. before usage.

Agar plates (5 plates for each organism per raw extract and replicated thrice) were inoculated with 0.1ml culture of the test organisms and spread with a glass rod shaped like a hockey stick and incubated at 30°C for 24 hours. Each plate was observed for zone of inhibition after inoculation.

Methods for phytochemical screening of leaves extract of Emilia sonchifolia

A phytochemical content was determined using standard procedures according to Association of Official Analytical Chemist of 2006.

Extraction of flavonoids

Two methods were used to test for flavonoids:

a. A portion of the leaf extract was heated with 10ml of ethyl acetate over a steam bath for 3 minutes, the mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution, a yellow coloration indicated the presence of flavonoids.
b. Dilute ammonia (5ml) was added: a yellow coloration indicated the presence of flavonoids.

Extraction of Alkaloids

Extracts was dissolved individually in dilute Hcl and filtered:

a. Filtrate was treated with Mayer's reagents (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids
b. Filtrate was treated with Dragendul's reagent (solution of potassium bismut iodide). Formation of red precipitate indicates the presence of alkaloids.
c. Filtrate was treated with Hager's reagent (saturated pricic acid solution) presence of alkaloid is confirmed by the formation of yellow colour precipitate.

Extraction of Saponins

0.5g of extract was added to 5ml of distilled water in a test tube and the solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Extraction of Triterpenoids

0.5g of the extract was added in 1ml of chloroform. 1ml of acetic anhydride was also added, followed by the addition of 2ml of concentrated H_2S_{O4}. Formation of reddish violet indicated the presences of triterpenoids.

Extraction of Tannins

Two methods were used to test for the presence of tannins in the leave extracts:

a) To 10ml of freshly prepared 10% KOH in a beaker, 0.5g of raw extract will be added and shaken to dissolve. A dirty precipitate observed indicated the presence of Tannins.
b) About 0.5g of the leaves extract was boiled in 10ml of water in a test tube and then filtered. A drop of 0.1% ferric chloride was added and the solution was observed for brownish green or a blue-black coloration.

Extraction of reducing sugar (Fehling's test)

0.5g of the leaves extract was dissolved in 5ml distilled water and filtered while hot. The filtrate was hydrolysed with dilute HCI, neutralize with alkali (NAOH) and heated with Fehling's A and B solutions. Formation of red precipitate indicated the presence of reducing sugar.

Extraction of anthraquinones

0.5g of the leaves extract was boiled with 10ml of H_2SO_4 and filtered. The filtrate was shaken with 5ml of chloroform, the chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour changes which indicated the presence of anthraquinones.

Extraction of steroids

0.5g of the leaves extract was dissolved in 10ml of chloroform and equal volume of concentrated H_2SO_4 was added by the sides of the test tubes. Reddish upper layer and yellowish sulphuric acid layer with green fluorescence indicated the presence of steroids.

Extraction of cardiac glycosides (Keller-Killian test)

To 0.5g of leaves extract dissolved in 5ml water was added to 2ml of glacial acetic solution containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated H_2SO_4. A brown ring at the interface l indicated the presence of deoxysugar characteristics of cardenolides. A violet ring appeared below the brown ring while the acetic acid layer a greenish ring was formed above the brown ring and gradually spread through this layer.

Statistical analysis

The experimental set up was a 5 x 5 factorial experiment in a simple completely randomised design with three

replications to give a total of seventy five (75) experimental units.

Factor 1 was the extract concentrations with 5 levels of 0%, 10%, 20%, 30% and 40%.

Factor 2 was the test organisms which were five (5) in number and included Shigella spp., Salmonella spp., Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus.

The experiment was replicated thrice to ensure repeated measurements and correctness of results. Zones of inhibition measured from each plate were collated and analysed using simple percentages.

RESULTS

Results of phytochemical screening and profiling of the leaves extract of Emilia sonchifolia had indicated that most of the phytochemicals are present in high concentrations, few others at low concentrations and steroids not present at all. These high concentrations of phytochemicals in the leaves extract of the plant possess high antioxidant and inhibitory effect to help to slow down the growth of the bacterial pathogens that triggers off diarrhoea in humans. The results of the presence of low and high concentration of these phytochemicals in the leaves extract of Emilia sonchifolia are presented in the table 1 below.

Table 1: Results of phytochemical contents of leaf extract of Yellow tassel (Emilia sonchifolia) flower.

Phytochemicals	Absent	Present
Anthraquinones		++
Tannins		+++
Triterpenoids		++
Saponins		++
Alkaloids		+
Flavonoids		++
Reducing Sugars		+
Cardiac gylcosides		++
Steroids	--	

- = Complete absence
+ = Low presence
++ = Moderately present
+++ = Highly present

Although many organisms have been implicated with diarrhoea, the present study was however limited to five (5) due to their high prevalence. The reaction of the different isolates based on the different status (diluted or undiluted) of the extracts was carefully evaluated and presented herewith as follows;

Table 2: Effect of undiluted (crude) extracts of Emilia sonchifolia leaves on five (5) isolated diarrhoeagenic pathogens.

Diarrhoeagenic Pathogens	Percent (%) of Undilution				
	Control (0)	10	20	30	40
Escherichia coli	S	S	S	MS	R
Shigella species	S	S	S	MS	R
Staphylococcus aureus	S	S	S	S	R
Salmonella species	S	S	MS	R	R
Pseudomonas aeruginosa	S	S	MS	R	R

In the undiluted extract of Emilia sonchifolia, Escherichia coli and Staphylococcus aureus showed a high sensitivity (S) of over 80% each with about 70% Inhibition (R), Shigella on the other hand showed 60% sensivity (S) with about 20% Moderately sensitive (MS) and 20% Inhibition (R) respectively. Salmonella species and Pseudomonas aeruginosa shows each 40% sensitivity (S), 20% moderately sensitive (MS) and 40% Inhibition (R).
R=Inhibition
MS=Moderately Sensitive
S=Sensitive

Table 3: Effect of diluted (crude) extracts of Emilia sonchifolia leaves on five (5) isolated diarrhoeagenic pathogens.

Diarrhoeagenic pathogens	Percent (%) of Dilution				
	Control (0)	10	20	30	40
Escherichia coli	S	S	S	MS	R
Shigella species	S	S	MS	R	R
Staphylococcus aureus	S	MS	MS	R	R
Salmonella species	S	MS	MS	MS	MS
Pseudomonas aeruginosa	S	S	R	R	R

In the diluted extract of Emilia sonchifolia, Escherichia coli showed 60% sensitivity(S) with 20% moderately sensitive (MS) and 20% inhibition (R). Shigella species and Pseudomonas aeruginosa each showed 40% sensitivity (S) against the diluted extract and 20% moderately sensitivity (MS) and 40% inhibition (R) while and Staphylococcus aureus showed 20% sensitivity (S) and 40% Inhibition. Salmonella species showed 20% sensitivity and 80% moderately sensitive (MS).
R= Inhibition
MS=Moderately Sensitive
S=Sensitive

DISCUSSION

The distribution of diarrhoeal parasites world-wide, is increasing from 5 % to 50% according to WHO reports of 2003 and Shylesh, 2005. The prevalence of the intestinal pathogenic bacteria parasite is the most common diagnosis among patients and tends to occur in high intensity among patients suffering from dysentery, cholera and diarrhoea (WHO, 2003).

Of the five isolated bacterial pathogens studied, the effect of aqueous extract of Emilia sonchifolia on the undiluted extracts, Escherichia coli and Staphylococcus aureus showed the highest sensitivity of over 90 % zone of inhibition each with about 10% resistance. Shigella species show sensitivity of 60% zone of inhibition and 20% resistance. Salmonella species and Pseudomonas aeruginosa each showed sensitivity of 40% inhibition zone, 20% moderate sensivity and 40% resistance. The high sensitivity exhibited by the various isolates on the plate disc loaded with undiluted extract can be attributable to the concentrated nature of the extract and also due to the dilution factor used for the study. The results of the present study which showed that leaves extracts of Emilia sonchifolia exhibits high efficacy in the inhibition of diarrhoeagenic pathogens growth in agar media is a confirmation of the earlier reports of Shylesh, (2005), who posited that various extracts of Emilia sonchifolia has a cytotoxic effect on disease causing pathogens such as Pseudomonas aeruginosa, Escherichia coli, Vibrio parahemolytica, Staphylococcus aureus and Shigella species. It implies that extracts of Emilia sonchifolia contains a high proportion of phytochemicals which are high ctyotoxic to the pathogenic organisms which can be harnessed and exploited for the production of pharmaceutical products for the treatment of diarrhoea, dysentery and other related illnesses of which diarrhoea remains a major symptom. Chopra and Major, (2006) in their investigations had also posited that leaves extracts of Emilia sonchifolia contains high proportion of tannins which have been very effective against pathogenic organisms implicated in various human ailments and diseases.

CONCLUSION

Presently, natural botanical extractions and their derivatives play an important role in drug discovery process of pharmaceutical industries. This research is to evaluate the efficacy of Emilia sonchifolia as an antibacterial agent and as an effective herbal therapy for the treatment of diarrhoea and other diseases associated with the causative pathogenic organisms. The results indicates that leaf extracts of Emilia sonchifolia can be harnessed and used in the formulation of medicinal therapy for the treatment of diarrhoea associated illnesses if adequate attention is given to this research findings.

REFERENCES

1. Ahmed, M.M., S.Qureshi, A., M. Albekeri and R.M.Rao Anti-Inflammatory activity of Caralluama tuberralata alcoholic extract, Pharmacological Research Journal, 1993; 2(1): 476 – 468.
2. Alter, R., M. Masima Akter., R Hasanur., A. Nazimuddin and I. Shahidw Analgesic and anti-inflammatory effect of whole Agerantum Conyzoides and Emilia sonchifolia alcoholic extracts in animal models. African Journal of pharmacy and pharmacology, 2012; 9(20): 1469-1476.
3. Arora, D.R and Arora B Medical parasitology. CBS publisher and Distributors, New Delhi, Bangloue, India, 2008; 3-5.
4. Association of Official Analytical Chemist (A.O.A.C). Procedures and Protocols for chemical analysis in samples, 2006.
5. Blanco, J. Blanco, M. Gonzalez, E. A Serotypes and colonization Factors of enterotoxigellic Escherichia

coli isolated in various countries. European Journal of Epidemiology, 1993; 9: 487-489.
6. Briana, L. Rammurthy, T., Soura S.,Yoshifumi, T., Kirishinan, R and Cohu, O Diarrheaogenic pathogens in polymiocrobial infections. Journal of pharmacology, 2011; 2(1): 187 – 193.
7. Cheng, D and Redder, E Pyrrolizidin alkaloids, Emilia sonchifolia. Plantation medicine, 2004; 6: 484-486.
8. Chopra, R.N and Major, S.L Glossary of Indian Medicinal plants (including the supplement). Council of scientific and industrial research New Delhi, 1996; 245 – 278.
9. Comfort, C.M and Ogbonnaya, A.E Effects of aqueous extracts of Emilia sonchifolia on liver enzyme in Dithizone induced diabetes in Rabbits. Nigeria Journal of animal research, 2000; 24(1): 8-15.
10. Couto, V., Vilela, F., Dias, D., Santos, M., Soricini, R and Giusti-palva, A Antinociceptive effect of extract of Emilia sonchifolia in mice. Journal of Ethnopharmacology, 2011; 134(2): 248-253.
11. Duke, J.A and E.S.Ayensu, Medicinal plant of China. Reference publications, ISBN 0-917256-20-4, 2005.
12. Essien, G.E., Nwidu, L and P.A. Nwafor Anti-inflammatory and analgesic potential of methanolic extract of Emilia sonchifolia (composistion Leaves in rodents. Journal of medicinal plants, 2009; 3(1): 45-50.
13. Etukudo, N.E and Inyang, A.N Forest, Our divine treasure. Dorand Publisher, Nigeria Ltd, 2001; 65-70.
14. Gayanthri, G.E Medicinal Value of Plants. Cambridge University press UK, 2012; 175-184.
15. Hasegawa, H Effect of extract on isolation of Pseudomonas species and Antifungal agents against phytopathogenic fungi. Journal of plant prot-ection, 1990; 2(1): 46 – 49.
16. Mamta, R. Natural antioxidants (flavones glycoside) from Emilia sonchifolia and its potential activity. International journal of pharmacy and pharmaceutical Sciences, 2012; 4: 45-52.
17. Mcfarl, W and Lynn, G Analysis of probiotics for the prevention of Travellers diarrhoea, travel medicine and infectious diseases. Journal of Epidemiology, 2007; 5(2): 97-105.
18. Muko, K.N. and Ohiri, F.C A preliminary study on the anti-inflammatory properties of Emilia sonchifolia leaf extracts. Phytoterapia, 2000; 71(1): 65-68.
19. Ramammurthy, D Emilia sonchifolia extracts on the control of diarrhoea. Journal of epidemiology, 2011; 2(1): 98-104.
20. Shylesh, M. Preliminary studies on the cytotoxic effect of various extracts of Emilia sonchifolia on phytopathogens. Phytoterapia, 2005; 3(2): 675-679.
21. World Health Organization Bulletin on the prevalence of intestinal Parasites information among diarrhoeal patients, 2003; 25-38.