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Original Article

Phytochemical and Synergistic activities of *Tapinanthusglobiferus* Growing on *Tarminalia Catappa* with Amoxicillin

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Abstract: When combined in a certain ratio, the effects of ampicillin and tapinanthusglobiferus demonstrated relevance in the treatment of diseases caused by Epulopiscium spp.The purpose of this study was to use broth microdilution and checkerboard techniques to examine the phytochemical content and assess the synergistic effects of amoxicillin and methanol leaf extract of T.globiferus grown on Tarminalia catappa against bacterial isolate.Amoxicillin demonstrated a minimum inhibitory concentration (MIC) of 10 mg/mL against the test organism, whereas T. globiferus displayed a MIC of 25 mg/mL. While ratios (6:4 and 5:5) with fractional inhibitory concentration (FIC) index 1.2 and 1.5 indicated indifference against the test organism, other ratios (9:1, 8:2, 7:3, 4:6, 3:7, 2:8, and 1:9) with fractional inhibitory concentration (FIC) index of 0.56, 0.60, 0.65, 0.8, 0.85, 0.9, and 0.95 demonstrated synergism upon combination. This study's findings indicated that certain ratio combinations may be indifferent or synergistic.Phenolic, saponnins, tannins, flavonoids, steroids, and terpenoids were found in the methanol leaf extracts of Tapinanthus globiferus growing on Tarminaliacat, according to a preliminary phytochemical screening. The plant has an antibacterial effect and can therefore be used to treat diseases caused by Epulopiscium spp. The minimum inhibitory concentration (MIC) of the extract revealed that the plant's methanol extract could inhibit the growth of Epulopiscium spp. at a concentration of 25 mg/ml.

Keywords: Phytochemical Screening; Synergy; Amoxicillin;*Tapinanthusglobiferus*.

I. INTRODUCTION

The leaves of the Combretaceous plant Terminaliacatappa L. are commonly used in Southeast Asian folk medicine to cure dermatosis and hepatitis. This species is found throughout the world, ranging from Australia to Indo-Maleisa. The big, spreading tropical almond tree, Terminalia catappa, is currently found in coastal regions across the tropics. The tree can withstand somewhat high salinity in the root zone, strong winds, and salt spray. It grows best in sandy, well-aerated soils that drain freely. Due to the species' extensive supply of non-wood goods and services, coastal communities have historically benefited greatly from it. It is essential for stabilising coastlines and has a fibrous, spreading root system. It is commonly planted for shade, ornamental uses, and edible nuts throughout the tropics, particularly near sandy seashores. The wood works well as a decorative and functional general-purpose hardwood, and it may be used to produce furniture and interior building materials. Fruits begin to develop around the age of three, and the pleasant and nutritious seed kernels can be consumed right away. Terminaliacatappa L. is its preferred scientific name. According to Mohale et al. (2009), it belongs to the Combretaceae (combretum family) family. [1].
Because of the adverse effects of antimicrobial agents and the rise in bacterial resistance, research has shifted in the last few decades towards herbal remedies. When combined with other antibiotics to treat pathogenic bacteria, the plant extract may boost its effectiveness (Shahabeet al., 2019).[2].In order to eradicate pathogenic microorganisms that infiltrate gingival tissue, antimicrobial treatments are essential. In clinical practice, the most commonly utilised supplementary therapies for the treatment of periodontitis problems are amoxicillin, metronidazole, tetracycline, azithromycin, and ciprofloxacin. A serious concern is the rapidly increasing number of multidrug-resistant bacteria that are resistant to current antibiotics. These bacteria are the main cause of treatment failure and are rising as a percentage of all deaths. Therefore, the development of antibacterial medicines that not only stop drug resistance but also enhance the outcome of treating infectious diseases becomes imperative (Shahabeet al., 2019).[2].Over time, the idea of combining two or more antibiotics has been explored as a way to overcome

resistance brought on by specific microbes. When treating bacterial infections, a high degree of synergistic impact could be attained. There are two methods for assessing medication interactions in vitro: the Checkerboard approach and the Time Killing Curve method.In this study, the checkerboard approach will be used.According to Ibezim et al. (2006), amoxicillin is a broad spectrum antibiotic that is effective against both Gram-positive and Gram-negative cocci, salmonella, intestinal bacilli, Shigella, listeria, and certain forms of hemophilic bacilli. [3].

The most prevalent mistletoe on Vitellariaparadoxa is Tapinanthusglobiferus, while Tamandus indica is a hemi-parasitic plant (Mukhtaret al., 2022).The species in question are Azadirachta indica (Haliluet al., 2016)[5], Ficus glumosa (Abubakaret al., 2017)[6] found in West Africa, and Watson (2001) notes that it is a primary cause of Vitellaria paradoxa mortality in the northern savannah boundary.[7]. Tapinanthusglobiferus, a member of the Loranthaceae family, is locally known as mistletoe (English), Kauchinkadanya (Hausa), Eme-emiafomo (Yoruba), and Osisi/Okwumaosa (Igbo) in Nigeria (Burkill, 2000)[8]. Tapinanthusglobiferus is a semi-parasite that mostly grows on the branches of many different tree species, including Vitellariaparadoxa, Kola, Citrus, Combretum, Acacia, Aloe, and Terminalia as host trees. It has glabrous pendulous stems up to 1.2 m long and likely roots (Waterberg et al., 1989).[9].Traditional herbalists in the area utilise tapinanthusglobiferus to treat a variety of illnesses, such as diabetes and stroke (Odugbemi, 2006).[10].

A lotion for the treatment of itching is made in Sudan using Tapinanthusglobiferus (Burkill, 2000).[8].Fresh Tapinanthusglobiferus, also known as hadhal in the region, is fed orally to all kinds of livestock in Saudi Arabia in order to alleviate fever and remove the placenta following parturition. In conventional medicine, tapinanthus globiferus is used to treat convulsions, ulcers, headaches, diabetes mellitus, malaria, stroke, and stomach issues (personal communication). According to reports, viscum album, often known as mistletoe, has several medicinal applications for treating a variety of conditions, including diabetes mellitus, stroke, gastrointestinal issues, heart palpitations, elevated blood pressure, and respiratory issues (Burkill, 2000).[8] Using the Checkerboard method, this study examined the phytochemical screening and in vitro synergistic effect of amoxicillin and methanol leaves extract of Tapinathus globiferus growing on Tarminalia catappa. The findings of this study will serve as a foundation for the use of standardised herbal preparations in combination therapy to treat infectious disorders with high rates of morbidity (Burkill, 2000). [8].

II. METHODOLOGY

A. Collection and Identification of Plant:

A voucher specimen number, PCG/UDUS/0009, was deposited with the Herbarium Unit of the Faculty of Pharmaceutical Sciences Department of Pharcognocy at Usmanu Danfodio University in Sokoto, where the plant material was taken from the Mabera Salame area.

B. Preparation of Plant Material:

The plant material was gathered, stored, and dried. Using a lab mortar and pestle, the dried material was ground up and kept in polythene bags for later use.

C. Preparation of the Methanol leaf Extract:

200 millilitres of methanol were macerated with 350 grammes of powdered plant material for a full day.After filtering the material, the filtrate was concentrated in a water bath to create a crude methanol extract, which was then employed for antibacterial research.

D. Phytochemical Screening:

T. globifeus crude methanol leaf extract was subjected to a preliminary phytochemical screening utilising conventional protocols in order to detect the presence of secondary metabolites (Trease and Evans). [11].

E. Preparation of Stock Solution of the Extract and amoxicillin:

Using an analytical weighing scale, one gramme of the extract was weighed and then dissolved in two millilitres of DMSO to create the stock solution, which had a 500 mg/mL concentration. A precise weight of 0.8 g of ciprofloxacin was obtained, and it was dissolved in 2 ml of distilled water to yield a 40 mg/mL stock solution.

F. Preparation of Culture Media:

The preparation of the culture medium followed the manufacturer's instructions. A weighed quantity of 23 g of Mueller Hinton broth was dissolved in 1000 mL of purified water. Up until it was totally dissolved, it was swirled. The media was autoclaved for 15 minutes at 121 degrees Celsius to sterilise it, and then it was cooled to room temperature.

G. Standardization and Culturing of the Bacteria Isolate:

The uniformity In accordance with Clinical Laboratory Standard criteria, epulopiscium spp. was isolated using normal saline and its turbidity was adjusted to meet 0.5 McFarland standard turbidity standards. [12].

H. Determination of Minimum Inhibitory Concentration (MIC):

Using Muller-Hinton Broth (MHB) medium against the test organisms, the MIC was ascertained by the broth macrodilution method, in accordance with the recommendations of the Clinical and Laboratory Standards (Spoorthiet al., 2011)[13]. After removing 1.6 mL of the extract from the stock solution and adding 2.4 mL of Mueller-Hinton broth to the first tube, a twofold serial dilution was performed, resulting in concentrations ranging from 200 mg/mL to 12.5 mg/mL.The organism was placed into each tube in an amount of around 0.1 mL. As a positive control, a tube with just the organism and the sterilised media was utilised. After that, the tubes were incubated at 37 °C, and turbidity was used as a criterion to determine whether or not growth was present. The minimum inhibitory concentration (MIC) of a serial dilution was defined as the lowest concentration that did not exhibit any obvious evidence of growth after 24 hours.

I. Evaluation of Combined Effects of Methanol Extract and Amoxicillin:

The stock solution of the extract (25 mg/mL) and ciprofloxacin (10 mg/mL) were produced in double strength Muller-Hinton broth and autoclaved at 121°C for 15 minutes, using the matching MIC found above. As previously mentioned by Spoorthiet al. (2011)[13], a continuous variation Checkerboard approach was used to prepare varying amounts of the Extract (E) and Amoxicillin (A).A 0.1 ml culture of 106 cfu/mL of the test microorganism was added to each portion of the herbal extract/amoxicillin combination, and it was then incubated for 24 hours at 37°C. By calculating the fractional inhibitory concentration (FIC) indices using the formulae below, the combined effect was evaluated algebraically (Michael 2010). [14]

FIC_{index} = FIC Extract + FIC Amoxicillin ..... equation (1)

FIC Extract (fractional inhibitory concentration of Extract)

$$= \frac{\text{MIC of Extract in combination with amoxicillin}}{\text{MIC of Extract alone}} \text{ ..... equation (2)}$$

FIC amoxicillin (fractional inhibitory concentration of amoxicillin)

$$= \frac{\text{MIC of amoxicillin in combination with Extract}}{\text{MIC of amoxicillin alone}} \text{ ..... equation (3).}$$

III. RESULTS AND DISCUSSION

A. Phytochemical Screening

Table 1: Result of the phytochemical screening of methanol leaves extract of *Tapinanthusglobiferus*growing on *Tarminaliacatappa*

Phytochemicals	Test	Inference
Alkaloids	Dragendoffs test	+
	Wagners test	N.D
Phenolic compounds	Ferric chloride test	+
Tannins	Ferric chloride test	+
Glycosides	Keller – killiani test	N.D
Saponins	Frothing test	+
Flavonoids	NaOH test	+
Steroids/Triterpenes	Salkowski test	+
	Lieberman Buchardtest	+

Key: *+ = Present, - = Not Detected*

Table 2: Result of the minimum inhibitory concentration of extract and Amoxicillin

Organism	Extract (mg/ml)	Ciprofloxacin(mg/mL)
Epulopisciumspp	25	10

Table 3: The result of combined effect of amoxicillinand *T. globiferus*

Ratio Extract/ Amoxicillin	MIC Extract (mg/mL)	MIC Amoxicillin (mg/mL)	FIC Extract	FIC Amoxicillin	FIC Index	Inference
10:0	25	-	-	-	-	-
9:1	11.5	1	0.45	0.1	0.55	SYN
8:2	10	2	0.40	0.2	0.60	SYN
7:3	8.75	3	0.35	0.3	0.65	SYN
6:4	7.5	4	0.30	0.8	1.10	IND
5:5	6.25	5	0.25	1.0	1.25	IND
4:6	5	6	0.20	0.6	0.80	SYN
3:7	3.75	7	0.15	0.7	0.85	SYN
2:8	2.5	8	0.10	0.8	0.90	SYN
1:9	1.25	9	0.05	0.9	0.95	SYN
0:10	-	10	-	-	-	-

Key: *MIC = minimum inhibitory concentration, AN =amoxicillin, FIC = fractional inhibitory concentration, SYN = Synergy, IND = indifference.*

The preliminary phytochemical screening of the methanol leave extracts of *Tapinanthusglobiferus* growing on *Terminaliacatappa*revealed the presence of phenolic compound,saponnins, tannins, flavonoids, steroids and terpenoids. This is in agreement with the work reported by (Emaikwu*et al* 2009)[15]who reported the phytochemicals constituent of methanol extract of *T. globiferus* to contain terpenoids, steroids, flavonoids, and tannins. This confirmed that the leave part of *Tapinanthusglobiferus*posseses antimicrobial properties as such, they can be used as drugs traditionally and also as lead. According to the report by some researchers conducted on *Tapinanthusglobiferus*species,the plant *T.globiferus* has maximum ability to synthesize secondary metabolites which serve as defensive mechanisms against microorganisms (Emaikwu*et al* 2009)[15].

Result of the minimum inhibitory concentration (MIC) of the extract showed that the methanol extract of the plant can inhibited the growth of *Salmonella typhi* at a concentration of 25 mg/ml, this showed that the plant have antibacterial effect and as such it can be used in the treatment of disease cause by *S. Typhi*.These findings are in line with the work of(Saravanan*et al.,* 2012)[16].Who reported the broad spectrum antibacterial activity of *T. globiferus*leaves against; *S. aureus*, *B. subtilis*, *E. coli* and *S. typhi* at dose dependent manner.

These observed activities may be due to the phytoconstituents present in the crude methanol extract of *T. globiferus*leaves (tannins, flavonoids, saponins etc. Tannins have reported to be able to interfere with the bacteria cell wall synthesis where they formed complex with polysaccharide through a non-specific forces such as hydrogen bonding and hydrogen effects as well as by covalent bond formation(Waseem. *et al.,* 2017)[17].
The non-significant findings with the extract at lower concentrations are in conformity with the work of (Shyamapada*et al* 2010)[18] who reported the broad spectrum antibacterial activity of *T. globiferus*against; *S. aureus*, *B. subtilis*, *E. coli* and *S. typhi*at dose dependent manner which increasewith increase in concentrations of the extract and decrease with decrease in the concentrationof the extracts.

The examination of the plant extract and ciprofloxacin's combined action revealed a significant effect at a certain proportion. Because combined antibiotic therapy offers a greater antibacterial spectrum, a synergistic impact, and a lower likelihood of resistance developing during therapy, it is preferable over monotherapy. (Bassey, 2012)[19]Synergy was defined as FIC index values less than 1, with the degree of synergy increasing as the value approaches zero. Additivity is indicated by a FIC index value of 1, indifference is indicated by a value larger than 1, but antagonism is indicated by a value greater than 2. Based on this, T. globiferus and amoxicillin together demonstrated synergistic action against S. Typhi in the following ratios: 9:1, 8:2, 7:3, 4:6, 3:7, 2:8, and 1:9; however, 5:6 and 5:5 demonstrated indifference.

IV. CONCLUSION

The finding from this study reveals that *Tapinanthusglobiferus*growing on *terminaliamantaly*contains some phytochemicals such as steroid, flavonoids, tannins, saponins and other phenolic compounds. The study also exhibited that the

plant has appreciable synergistic effects with amoxicillin against *Epulopisciumspp*, hence encourage the use of the plant extract in combination with amoxicillin for the management of diseases cause by *Epulopiscium spp*.

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