Sahel Journal of Life Sciences FUDMA (SAJOLS)
June 2024 Vol. 2(2): 1-7
ISSN: 3027-0456 (Print)
ISSN: 1595-5915(Online)
DOI: *https://doi.org/10.33003/sajols-2024-0202-01*

Research Article

Phytochemical Screening and Antibacterial Activity of Whole Plant Crude Extracts of *Euphorbia hirta* Linn against Some Clinical Isolates

***Oloninefa, S. D.[1], Aisoni, J. E.[2], Fadayomi, V. K.[3], Oghenemaro, O.[4]**

[1] Department of Biological Sciences, Kogi State University, Kabba, Nigeria
[2]Department of Microbiology, Bayero University, Kano, Nigeria
[3]Department of Microbiology, Federal University, Lafia, Nigeria
[4]Industrial Scientific Nutritional (ISN), Abuja, Nigeria

***Corresponding Author:** *sdoloninefa@ksukabba.edu.ng*; Tel.: 08038893341

ABSTRACT

The problem of bacteria resistance coupled with microorganisms (e.g. Bacteria, fungi, viruses and protozoa) been implicated as causative agents of many infectious diseases has led many researchers globally to search for substances (Phytochemicals) which can be used to stop the activity of these microorganisms and to reduce or eliminate their effect on the human population and to also curb the menace of bacteria resistance. This study aimed at carrying out phytochemical screening and antibacterial activity of whole plant crude extracts of *Euphorbia hirta* Linn against some clinical isolates. The selected clinical isolates (*Escherichia coli*, *Pseudomonas fluorescens*, *Klebsiella pneumoniae*, *Salmonella typhimurium*) were identified using biochemical tests. The extraction of whole plant of *Euphorbia hirta* with *n*-hexane, ethyl acetate, methanol and water was done using reflux extraction technique. The screening for phytochemicals was carried out using standard methods while the determination of antibacterial activity of the extracts against selected clinical isolates was carried out using agar well diffusion method. The results obtained show that phytochemicals such as: flavonoids, terpenoids, alkaloids, steroids, tannins, saponins, phenols, reducing sugars were present while cardiac glycosides was absent in all the extracts. The highest (21.33±0.33) antibacterial activity which is concentration-depended was recorded against *Pseudomonas fluorescens* using ethyl acetate extract while *n*-hexane extract has no antibacterial activity against any of the clinical isolates which possibly may be due to insufficient bioactive agents in the *n*-hexane extract. The study revealed that *Euphorbia hirta* contained phytochemicals which have antibacterial activity and can be very useful in drug development, traditional and complementary medicine if utilized.

Keywords: Phytochemical screening, Antibacterial activity, *Euphorbia hirta*, Traditional medicine, Clinical isolates

Citation: Oloninefa, S.D., Aisoni, J.E., Fadayomi, V.K., Oghenemaro, O. (2024). Phytochemical Screening and Antibacterial Activity of Whole Plant Crude Extracts of *Euphorbia hirta* Linn against Some Clinical Isolates. *Sahel Journal of Life Sciences FUDMA*, 2(2): 1-7. DOI: *https://doi.org/10.33003/sajols-2024-0202-01*

INTRODUCTION

Euphorbia hirta Linn is a weed plant of the tropics and subtropics that belong to the family of *Euphorbiaceae*. It is commonly referred to as milkweed (Dudhy) and an asthma plant and known by different names in different parts of the world (Ghosh *et al*., 2018; Ghosh *et al*., 2019; Tripathi *et al*., 2021). It is found throughout tropical Africa, India, China, Japan, Taiwan and neighboring islands as well as in the islands of the southern Pacific. According to Pranabesh *et al*. (2019), it grows in continental tropical America and the Caribbean. It is an annual broad-leaved herb that has hairy stem, usually erect, slender stemmed; spreading up to 45 cm tall though sometimes can be seen lying down with many branches from the base to the top and the stem and leaves produce white or milky juice when cut as reported in the works of Tuhin *et al*. (2017) and Panzu *et al*. (2020). As reported by Asha *et al*. (2016)

and Gokulprasath *et al*. (2021), the usefulness of *E*. *hirta* in traditional and complementary medicine cannot be over emphasized. It is used to cure pimples, female disorders, respiratory ailments (cough, asthma, coryza and bronchitis), dysentery and tumors (Kausa *et al*., 2016). *E. hirta* contains phytochemicals such as: triterpenoids, sterols, alkaloids, glycosides, flavonoids, tannins, phenols, choline and shikimic acid while some of the reported activities include its use as an antispasmodic, antiasthmatic, expectorant, anticatarrhal, antisyphilitic, antibacterial, antidiabetic, antivenom, wound healing, antimalarial, immunostimulatory, antithrombocytopenic, antioxidant, anticancer, antiallergy, anti-inflammatory and diuretic (Gopi *et al*., 2016; Kanedi, 2017; Anjum *et al*., 2017; Kikete *et al*., 2018; Thabet *et al*, 2018; Aleksandrov *et al*., 2019; Rahman *et al*., 2019; Uzor, 2020; Mahabati *et al*., 2020; Tran *et al*., 2020; Gokulprasath *et al*., 2021). However, El-Mahmood (2009) reported that *E. hirta* is a very popular herb amongst practitioners of traditional medicine and some of its local names in Nigeria include "*nonon furchiya*" in Hausa, "*tepel*" in fulzfulde, "*Harvom*" in Kaka and "*Hammock sand mat*" (Florida) while the exudate of the stem is used to treat eye and ear infections in Nigeria. This study focuses on phytochemical screening and antibacterial activity of whole plant crude extracts of *Euphorbia hirta*.

MATERIALS AND METHODS

Collection of Samples

Plant material

Fresh whole plant of *Euphorbia hirta* were collected around house-holds in Sauka-Kahuta area of Minna, Niger State, Nigeria and taken to the Department of Biological Sciences in Federal University of Technology, Minna for botanical identification. The identified plants were authenticated by Department of National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja. Nigeria with the voucher number, NIPRD/H/6864 which was then deposited in the same Herbarium Department of the Institute.

Clinical Isolates

The *Escherichia coli*, *Pseudomonas fluorescens*, *Klebsiella pneumoniae*, *Salmonella typhimurium* used were collected from the Microbiology Department of General Hospital, Minna.

Identification of the Clinical Isolates

The identification of the clinical isolates were carried out by Gram reaction and biochemical tests (such as catalase, citrate utilization, methyl red, indole, urease and triple sugar iron) as outlined in Cheesbrough (2010) and then sub cultured into Nutrient Agar slants, Eosin Methylene Blue Agar slant and Deoxycholate Agar slant and kept in a refrigerator until they were ready for further use.

Preparation of Extracts

The identified *E. hirta* was thoroughly washed with water to remove any extraneous substances and dirts and then dried at room temperature away from sunlight and dust. The air-dried whole plant were grounded to powder using Panasonic electric blender model MX-J210PN. The extraction was done using reflux extraction method as described in the work of Oloninefa *et al*. (2018).

Phytochemical Screening of the Crude Extracts

The crude extracts were subjected to phytochemical analysis to screen for the presence of bioactive components such as alkaloids, reducing sugar, saponins, flavonoids, cardiac glycosides, terpenoids, steroids and tannins. The phytochemical screening was carried out using standard procedure as described in the study by Bandiola (2018).

Antibacterial Activity of the Crude Extracts

Standardization of clinical isolates

The population of the clinical isolates were determined from the 0.5 McFarland Turbidity Standard prepared (Murray *et al*., 2007).

Preparation of extract concentration

The extract concentration was prepared as described in the work of Oloninefa *et al*. (2018). Two hundred milligram (200 mg) of the *n*-hexane, ethyl acetate, methanol and aqueous crude extract were weighed in 5 ml each of 20% Dimethyl sulfoxide (DMSO) (20 ml DMSO was made up to 100 ml with distilled water) to give 40 mg/ml concentrations respectively. The concentrations of 60 mg/ml; 80 mg/ml and 100 mg/ml were prepared following the same procedure.

Determination of the antibacterial activity of the crude extracts

The antibacterial activity of the crude extract was carried out using Agar Well Diffusion method outlined in CLSI (2015). Mueller Hinton Agar (MHA) was prepared and sterilized according to manufacturer's instruction. Petri dishes containing about 20 ml MHA were streaked with standardized 24 h culture of the clinical isolates using sterilized wire inoculating loop. Wells were cut with a 6

mm sterile cork borer and then sealed at the bottom with a drop of molten agar so as to prevent the extract from sipping beneath the agar. Four holes were made on each plate and adequately spaced out. About 100 µl of each of the crude extracts (40, 60, 80 and 100 mg/ml) (namely aqueous, methanol, *n*-hexane and ethyl acetate extracts) was delivered into each well and 40 mg/ml of the standard drug (Ciprofloxacin) were used for positive control while dimethylsulfuroxide (DMSO) served as the negative control. One-hour pre-diffusion time was allowed after which the plates were incubated at 37^{o}C for 24 h. The zones of inhibition were measured by direct linear measurement using a meter scale rule. The above method was carried out in triplicates and the mean of the triplicate result were taken.

Data Analysis

The analysis of variance (ANOVA) of the values obtained for the antibacterial susceptibility was carried out using IBM SPSS Statistics Version 23. All data were expressed as mean ± standard error of the mean. The values with different superscripts along the same column were significantly different ($P < 0.05$).

RESULTS

Gram Reaction and Biochemical Characteristics of the Clinical Isolates

The findings from the clinical isolates tested revealed the presence of *E. coli, S. typhimurium, K. pneumoniae and P. flourescens* from (Table 1).

Phytochemical Components in Whole Plant Crude Extracts of *Euphorbia hirta*

The phytochemical screening of the whole plant extract shows some bioactive components. The results showed that flavonoids, terpenoids, alkaloids, steroids, tannins and phenols were present in *n*-hexane crude extract (NHE) whereas cardiac glycosides, saponins and reducing sugars were absent. On the other hand, alkaloids, flavonoids, phenols, tannins, terpenoids, redu]]cing sugars and steroids were present in ethyl acetate crude extract (EAE) but saponins and cardiac glycosides were absent. Meanwhile, saponins, flavonoids, terpenoids, tannins, phenols, alkaloids, steroids and reducing sugars were present in methanol extract (ME) whereas cardiac glycosides was absent. The aqueous crude extract (AQE) had saponins, flavonoids, terpenoids, tannins, phenols and alkaloids while cardiac glycosides and steroids were absent (Table 2).

Antibacterial Activity of Whole Plant Crude Extracts

The *n*-Hexane Extract (NHE) had no antibacterial activity against all the isolates. The antibacterial activity results of Ethyl Acetate Extract (EAE) against the isolates were as follows: *S. typhimurium* (11.67-15.67); *K. pneumoniae* (10.33-12.00) and *P. fluorescens* (19.67-21.33) while methanol extract (ME) had antibacterial activity against *P. fluorescens* (16.00-17.33). More so, the antibacterial activity results of aqueous extract (AQE) were as follows: *E. coli* (11.67); *S. typhimurium* (9.00-13.00); *K. pneumoniae* (9.67-12.67) and *P. fluorescens* (10.67). However, Ciprofloxacin had antibacterial activity against the isolates as follows: *E. coli* (11.67-49.67); *S. typhimurium* (45.33-48.67); *K. pneumoniae* (50.00-55.00) and *P. fluorescens* (34.67-38.33). The DMSO had no antibacterial activity against all the isolates. The results are shown in Tables 3 and 4.

Table 1: Gram Reaction and Biochemical Characteristics of the Clinical Isolates

Tests	A	B	C	D
Gram Reactions	-	-	-	-
TSI	A/A, G (+), H_2S (-)	K/A, G (+), H_2S (+)	A/A, G (+), H_2S (-)	K/K, G (-), H_2S (-)
Methyl Red	+	-	+	-
Indole	+	-	-	-
Catalase	+	+	+	+
Citrate Utilisation	-	+	-	+
Urease	+	+	-	+
Identity	*E. coli*	*S. typhimurium*	*K. pneumoniae*	*P. fluorescens*

KEY: + = Positive; - = Negative; TSI= Triple Sugar Iron; K= Alkaline; A= Acid; G= Gas; H_2S= Hydrogen Sulphide

Table 2: Phytochemicals Components in Whole Plant Crude Extracts of *Euphorbia hirta*

Extracts	Saponins	Flavonoids	Terpenoids	Cardiac Glycosides	Tannins	Phenols	Alkaloids	Steroids	Reducing Sugars
***n*-hexane**	-	+	+	-	+	+	+	+	-
Ethyl acetate	-	+	+	-	+	+	+	+	+
Methanol	+	+	+	-	+	+	+	+	+
Aqueous	+	+	+	-	+	+	+	-	+

KEY: +: Present; - : Absent

Table 3: Antibacterial Activity of Whole Plant Crude Extracts of *Euphorbia hirta* (mm)

	Escherichia coli				*Salmonella typhimurium*			
Extracts/Control	**40 mg/ml**	**60 mg/ml**	**80 mg/ml**	**100 mg/ml**	**40 mg/ml**	**60 mg/ml**	**80 mg/ml**	**100 mg/ml**
n-Hexane	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Ethyl Acetate	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	14.67±0.33^{c}	11.67±0.88^{c}	13.33±0.33^{c}	13.67±0.33^{c}	15.67±0.33^{c}
Methanol	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Aqueous	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	11.67±0.33^{b}	9.00±1.15^{b}	11.67±0.33^{b}	12.33±0.33^{b}	13.00±0.58^{b}
Ciprofloxacin	11.67±0.33^{b}	44.33±0.67^{b}	46.00±0.58^{a}	49.67±0.33^{d}	45.33±0.33^{d}	46.00±0.58^{d}	48.00±0.58^{d}	48.67±0.33^{d}
DMSO	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Results represent mean ± standard error of mean of triplicate determination. Values with the same superscript in the same column are not significantly different at p<0.05

Table 4: Antibacterial Activity of Whole Plant Crude Extracts of *Euphorbia hirta* (mm)

	Klebsiella pneumoniae				*Pseudomonas fluorescens*			
Extracts/Control	**40 mg/ml**	**60 mg/ml**	**80 mg/ml**	**100 mg/ml**	**40 mg/ml**	**60 mg/ml**	**80 mg/ml**	**100 mg/ml**
n-Hexane	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Ethyl Acetate	10.33±0.67^{b}	10.67±0.67^{b}	11.33±0.88^{b}	12.00±0.58^{b}	19.67±0.33^{c}	20.00±0.58^{c}	21.00±0.58^{c}	21.33±0.33^{d}
Methanol	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	16.00±0.58^{b}	16.33±0.33^{b}	17.00±0.58^{b}	17.33±0.33^{c}
Aqueous	9.67±0.88^{b}	10.00±0.58^{b}	11.67±0.67^{b}	12.67±0.33^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	10.67±0.33^{b}
Ciprofloxacin	50.00±0.00^{c}	52.00±0.58^{c}	53.00±0.33^{c}	55.00±0.58^{c}	34.67±0.33^{d}	35.00±0.58^{d}	37.00±0.58^{d}	38.33±0.67^{e}
DMSO	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Results represent mean ± standard error of mean of triplicate determination. Values with the same superscript in the same column are not significantly different at p<0.0

DISCUSSION

The presence of phytochemicals such as saponins, flavonoids, terpenoids, tannins, phenols, alkaloids, steroids and reducing sugars in the whole plant crude extracts of *Euphorbia hirta* were in agreement with the previous studies by Abalaka *et al*. (2016); Ewansiha *et al*. (2016); Mekam *et al*. (2019); Haleshappa *et al*. (2020); Nirmal *et al*. (2020) and Vadalia *et al*. (2020) but the results disagreed with work of Awomukwu *et al*. (2014) because terpenoids and steroids were absent in the study. The difference in results may possibly be due to the type of solvents used for the extraction and the difference in the geographical location where the plants were collected (Ughachukwu *et al*., 2014). Abalaka *et al*. (2016) and Oloninefa *et al*. (2018) opined in their previous studies that phytochemicals have antimicrobial effects and as such they can be used for the treatment of diseases. In addition, Mann *et al*. (2014) Haleshappa *et al*. (2020) and Nirmal *et al*. (2020) revealed in their studies that the presence of the phytochemical components in the crude extracts are responsible for their effectiveness against many microbes and also enable the plant parts to function as herbs or drugs by producing biological activity in animals and in humans.

Furthermore, the results of antibacterial activity of the whole plant crude extracts of *E. hirta* showed that *n*-hexane extract (NHE) had no antibacterial activity when tested against all the clinical isolates. This may possibly be due to insufficient bioactive components in the NHE to effect antibacterial activity against the clinical isolates. Similarly, the four crude extracts had no antibacterial activity against *E. coli* at 40 mg/ml. This result is not in agreement with the previous studies carried out by Nikunj and Kaushik (2014); Jakhar and Dahiya (2017) and Nazeer (2017) that recorded antibacterial activity with all the crude extracts used in their studies. The variation in results obtained in this study may possibly be due to the solvent used for extraction, geographical location of the plant and the characteristics of the soil as opined by Ughachukwu *et al*. (2014).

The methanol extract (ME) had no antibacterial activity when tested against *E. coli*, *S. typhimurium* and *K. pneumoniae*. This result did not agree with the work of Nazeer (2017). This may likely be due to the clinical isolates used and insufficient bioactive components in the ME to cause antibacterial activity.

In addition, it was observed that ethyl acetate extract (EAE) had the highest zones of inhibitions with *P. fluorescens* compared with other crude extracts. This result did not agree with the work of Jakhar and Dahiya (2017) that recorded highest activity with *Klebsiella pneumoniae*. The use of different crude extracts and the isolates may likely be responsible for this result. More so, the values obtained for antibacterial activity of the crude extracts against the clinical isolates showed a significant difference at $p<0.05$. However, there was increment in the zone of inhibitions as the concentration of Ciprofloxacin (positive control) increases from 40-100 mg/ml while the DMSO (negative control) does not contain antibacterial agent hence the results obtained were expected.

CONCLUSION

The study revealed that the whole plant crude extracts of *Euphorbia hirta* contained phytochemicals which can be very useful in traditional and complementary medicine. The antibacterial activities of the crude extracts against the selected clinical isolates which results from the study revealed to be concentration-depended will also go a long way to significantly reduce even though does not eradicate microorganisms which have been implicated as causative agents of many infectious diseases. The results from this study show that crude extracts obtained from *Euphorbia hirta* Linn are very promising and can be used for drug development in pharmaceutical industries in the nearest future.

CONFLICT OF INTEREST

There is no conflict of interest among the authors.

REFERENCES

Abalaka, M. E., Akpor, O. B., Bello, A. B., Olonitola, O. S., Sani, A. and Osemwegi, O. O. (2016). Synthesis of Silver Nanoparticles (AgNPs) from Bark and Root of African Mahogany (*Khaya Senegalensis*) and The Comparative Studies of their Antimicrobial Properties. *Asian Journal of Microbiology and Biotechnology*, 1 (1): 25-32.

Aleksandrov, M., Maksimova,V. and Koleva Gudeva, L. (2019). Review of the anticancer and cytotoxic activity of some species from genus Euphorbia. *Agriculturae Conspectus Scientificusi.*, 84 (1): 1-5.

Anjum, V., Arora, P., Ansari, S. H., Najmi, A. K. and Ahmad, S. (2017). Antithrombocytopenic and immunomodulatory potential of metabolically characterized aqueous extract of *Carica papaya* leaves. *Pharmaceutical biology*, 55 (1): 2043-2056.

Asha, S., Thirunavukkarasu, P., Mani,V. M. and Sadiq, A. M. (2016). Antioxidant activity of *Euphorbia hirta* Linn leaves extracts. *European Journal of Medicinal Plants*, **7**: 1-14.

Awomukwu, D. A., Nyananyo, B. L., Onukwube, N. D., Uka, C. J., Okeke, C. U. and Ikpeama, A. I. (2014). Comparative Phytochemical Constituents and Pharmacognistic Importance of the Vegetative Organs of Some *Phyllanthus* Species in South Eastern Nigeria. *International Journal of Modern Botany*, 4 (2): 29-39.

Bandiola, T. M. B. (2018). Extraction and Qualitative Phytochemical Screening of Medicinal Plants: A Brief Summary *Int J Pharm*, 8 (1): 137-143.

Cheesbrough, M. (2010). Laboratory Manual. In: *District Laboratory Practice in Tropical Countries*. Cambridge University Press, United Kingdom, pp.146-157.

Clinical Laboratory Standards Institute (2015). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI document M100- S25, Volume 35, No. 3950 West Valley Road, Suite 2500 Wayne, Pennsylvania 19087, USA.

El-Mahmood, M. A. (2009). Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. *Journal of Medicinal Plants Research*, 3 (7): 498-505.

Ewansiha, J. U., Garba, S. A., Musa, G., Daniyan, S. Y., Busari, M. B., Innalegwu, D. A. and Doughari, J. H. (2016). Preliminary Phytochemical and Antimicrobial Activity of Citrus x limon (L) Burm. f. (lemon) Leaf Extract against Some Pathogenic Microorganisms. *Journal of Applied Life Sciences International*, 6 (4): 1-10.

Ghosh, P., Chatterjee, S., Das, P., Karmakar, S. and Mahapatra, S. (2019). Natural habitat, phytochemistry and pharmacological properties of a medicinal weed - Cleome Rutidosperma DC. (Cleomaceae): A comprehensive review. *International Journal of Pharmaceutical Sciences and Research*, 10 (4): 1000-08.

Ghosh, P., Das, P., Das, C., Mahapatra, S. and Chatterjee S. (2018). Morphological characteristics and phyto-pharmacological detailing of Hatishur (Heliotropium Indicum Linn.): A concise review. *Journal of Pharmacognosy and Phytochemistry*, 7 (5): 1900-07.

Gokulprasath, M., Lavanya, K., Ragul, G., Ranjithkumar, S. and Radha, P. (2021). Characteristics of *Euphorbia hirta*: A Review. *Journal of Emerging Technologies and Innovative Research*, 8 (12): 664-673.

Gopi, K., Renu, K., Vishwanath, B. S. and Jayaraman, G. (2015). Protective effect of *Euphorbia hirta* and its components against snake venom induced lethality. *Journal of ethnopharmacology*, 165:180-190**.**

Haleshappa, R., Keshamma, E., Girija, C. R., Thanmayi, M., Nagesh, C. G., Lubna Fahmeen, G. H., Lavanya, M. and Patil, J. S. (2020). Phytochemical study and antioxidant properties of ethanolic extracts of *Euphorbia milii*. *Asian Journal of Biological Sciences*, 13: 77-82.

Jakhar, S. and Dahiya, P. (2017). Antibacterial, Antioxidant Activity and Phytochemical Analysis of *Euphorbia hirta* Linn*. Microbiology Research Journal International*, 20 (2): 1-12.

Kanedi, M. (2017). Diueretic Activity of Plant Extract of Garden Spurge. Diueretic Activity of Plant Extract of Garden Spurge (*Euphorbia hirta L*.) on Male Wistar Rats. *European Journal of Biomedical and Pharmaceutical Sciences*, 4 (1): 516-519.

Kausa, J., Muthumani, D., Hedina, A. and Anand, V. (2016). Review of the phytochemical and pharmacological activities of *Euphorbia hirta Linn*. *Pharmacognosy Journal*, 8 (4): 310-313.

Kikete, S., Luo, L., .Jia, B., Wang, L., Ondieki, G. and Bian, Y. (2018). Plant-derived polysaccharides activate dendritic cell-based anti-cancer immunity. *Cytotechnology*, 70: 1097-1110.

Mahabati, M., Mahabati, A., Yuan, P., Liu, X., Cai, S., Aimaier, A., Ziyayiding, D., Yasheng, M., Abdujilile, D. and Li, J. (2020). Comparison of structural characteristics and immununoregulatory activities of polysaccharides from four natural plants. *Food and agricultural Immunology*, 31 (1): 545-562.

Mann, A. and Kuta, Y. A. (2014). Antibacterial activity of methanolic extracts of *Terminalia avicennioides* against fish pathogenic bacteria. *American Journal of Research Communication*, 2(4): 133-146.

Mekam, P. N., Martini, S., Nguefack, J., Tagliazucchi, D. and Stefani, E. (2019). Phenolic compounds profile of water and ethanol extracts of *Euphorbia hirta* L. leaves showing antioxidant and antifungal properties. *South African Journal of Botany*, 1 (1): 319-32.

Murray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. L. and Pfaller, M. A. (2007). Manual of Clinical Microbiology, 9th edn. ASM Press, Washington DC, pp. 2082-2091.

Nazeer, B. S. (2017). Antimicrobial Activity of *Euphorbia hirta* L. *Indian Journal of Research*, 6 (1): 1-2.

Nikunj, B. P. and Kaushik, C. P. (2014). Antibacterial Activity of *Euphorbia hirta* L. Ethanomedicinal Plant against Gram Negative UTI Pathogens. *International*

Journal of Pharmaceutical Research and Allied Sciences, 3 (2): 24-29.

Nirmal, C. R., Ebenezer, R. S., Kannan, P., Balasubramanian, M., Thirunavukkarasu, I., Mondal, R. and Dusthackeer, A. (2020). Anti-tuberculosis activity of bio-active compounds from Lantana camara L. *Euphorbia hirta* L. Mukia maderaspatana L. M. Roem and *Abutilon indicum* L. *European Journal of Integrative Medicine*, 35 (1): 101-105.

Oloninefa, S. D., Abalaka, M. E., Daniyan, S. Y. and Mann, A. (2018). Phytochemical Screening and Antibacterial Susceptibility of Whole Plant of *Euphorbia Heterophylla* Crude Extracts against Selected Bacteria Pathogens. *Bayero Journal of Pure and Applied Sciences,* 11(1): 211 – 220.

Oloninefa, S. D., Abalaka, M. E., Daniyan, S. Y. and Mann, A. (2022). Characterization and rate of killing of conjugated silver nanoparticles against selected clinical bacterial isolates. *Natural Resources for Human Health*, 2 (3): 343-348.

Palaniswamy, R. and Dhanyasri, S. (2019). Application of Endophytic Bacteria in Plant Growth Enhancement. *International Journal of Pharmacy and Biological sciences*, 9 (3): 457-461.

Panzu, N. N., Liyongo, C. I., Koto-te-Nyiwa, N., Florent Mukeba Biduaya, F. M., Kitadi, J.M., Kalulu, T., Mavinga, B. M., Dinangai, D. T., Kayembe, J. P. K. and Tshimankinda, P. M. (2019). Review on the phytochemistry, toxicology and bioactivities of *Euphorbia hirta* L.: A potential antisickling medicinal plant species. *Journal of Medicinal plant and Herbal Therapy Research*, 7: 8-18.

Pranabesh, G., Chandreyi, G., Shaktijit, D., Chandrima, D., Suprodip, M. and Sirshendu, C. (2019). Botanical Description, Phytochemical constituents and pharmacological properties of *Euphorbia hirta* Linn: A Review. *International Journal of Health Sciences and Research*, 9 (3): 273-286.

Rahman, M. S., Rana, S. and Islam, A. A. (2019). Antithrombotic and anti-inflammatory activities of leaf methanolic extract of *Euphorbia hirta Lin*. *International Journal of Complementary & Alternative Medicine*, 12 (4): 154-62.

Thabet, A. A.,Youssef, F. S., Korinek, M., Chang, F. R.,Wu, Y. C., Chen, B. H. and Hwang, T. L. (2018). Study of the antiallergic and anti-inflammatory activity of Brachychiton rupestris and Brachychiton discolor leaves (Malvaceae) using in vitro models. *BMC complementary and alternative medicine,* 18 (1): 1-15.

Tran, N., Nguyen, M., Le, K. P., Nguyen, N., Tran, Q. and Le, L. (2020). Screening of Antibacterial Activity, Antioxidant Activity, and Anticancer Activity of *Euphorbia hirta Linn*. Extracts. Applied Sciences, 10 (23): 1-15.

Tripathi, A. N., Sati, S. C. and Kumar, P. (2021). *Euphorbia hirta* Linn - An Invasive Plant: A Review of Its Traditional Uses, Phytochemistry and Pharmacological Properties. *International Journal of Pharmaceutical Sciences and Research*, 12 (12): 6189-6201.

Tuhin, R. H., Begum, M. M., Rahman, M. S., Karim, R., Begum, T., Ahmed, S. U., Mostofa, R., Hossain, A., Abdel-Daim, M. and Begum, R. (2017). Wound healing effect of *Euphorbia hirta* linn. Euphorbiaceae in alloxan induced diabetic rats. *BMC Complementary and Alternative Medicine*, 17 (1): 1-4.

Ughachukwu, P. O., Ezenyeaku, C. C. T., Ochiogu, B. C., Ezeagwuna, D. A. and Anahalu, I. C. (2014). Evaluation of antibacterial activities of *Euphorbia heterophylla*. *IOSR Journal of Dental and Medical Sciences*, 13 (11): 69-75.

Uzor, P. F. (2020). Alkaloids from plants with antimalarial activity: a review of recent studies. *Evidence-Based Complementary and Alternative Medicine*, 2020: 1-17

Vadalia, J. M., Sanandia, J. and Sheth, N. (2020). Comparative quantitative phytochemical and HPTLC analysis of two Euphorbiaceae family plants under the name Dugdhika. *J of Planar Chromatogr Modern TLC*, 33 (5): 473-9.

Watson, G.W. and Kubiriba, J. (2005). Identification of mealybugs (Hemiptera: Pseudococcidae) on banana *Musa sapientum* (L.) and plantain *Musa paradisiaca* (L.) in Africa. *African Entomology* 13(1): 35-47.

Wilson, A.C.C., Sternberg, L.S.L. and Hurley, K.B. (2011). Aphids alter host-plant nitrogen isotope fractionation. *Proceedings of the National Academy of Science* USA 108, 10220-10224.

Zakka, U. (2019). Proximate composition of Stored Dates (*Phoenix dactylifera* L.) infested with *Oryzaephilus surinamensis* (L.) *International Journal of Science and Research*, 6(11): 1958-1963.