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Phytochemical and antimicrobial activity of *Cascabela thevetia* seed oil L. LIPPOLD (Yellow Oleander)

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Abstract

This study analysed the phytochemicals and antimicrobial activity of oil extracted from seeds of *Cascabela thevetia.* Six phytochemicals were analysed. This includes Alkaloids, Flavonoids, Glycosides, Phytates, Saponins and Tannins. With Alkaloids and Glycosides to be slightly present. Flavonoids are moderately present while Phytates, Saponins and Tannins to be highly present. The quantitative results show 0.062 ± 0.001; 3.123 ± 0.005; 0.163 ± 0.010; 4.530 ± 0.01; 2.920 ± 0.010 and 3.140 ± 0.014 mg/g for Alkaloids, Flavonoids, Glycosides, Phytates, Saponins and Tannins respectively. *C. thevetia* oil showed a good degree of antimicrobial activities using the agar well diffusion method for gram-negative, gram-positive and fungi. *Staphylococcus aureus* a gram-positive bacterial, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* gram-negative bacterial shows zones of inhibition of 15.33, 12.00, 16.67 and 16.00 mm respectively. The fungi showed zone of inhibition of 22 mm for both *Rhizopus stolonifer* and *Candida albicans*. The MIC value for bacterial was obtained at 12.5 mg/ml while for the fungi the MIC was 6.25 mg/ml. *Pseudomonas aeruginosa, Salmonella typhi* and *Escherichia coli* MBC value was obtained at 50 mg/ml. *Staphylococcus aureus* have MBC value of 25 mg/ml and MFC value of 25 mg/ml was obtained for *Rhizopus stolonifer* and *Candida albican.*

Keywords: *Cascabela thevetia*; Phytochemicals; Antimicrobial; Grampositive bacteria; Gramnegative bacteria; Fungi

1. Introduction

Plants are essential in human activities both in the area of food and industries, hence the need for more and special research in the world of plants. The plant of interest in this research is *Cascabela thevetia.* *Cascabela thevetia* is a robust, evergreen shrub of up to 3-8m height. It is generally called yellow oleander in english, it belongs to the family Apocyanaceae, Genus *Cascabela*, species *Cascabela thevetia*. (Rojas-sandoval J, 2020). The plant is widely cultivated as an ornamental plant. The plants are found in many countries especially tropical areas like Sri Lanka, India, Africa and are native to southern Mexicans. The plants were said to originate from Mexico, central and south America and was later distributed to Hawii, United State, Texas, India, China and African. (Plants.ces.ncsu.edu, 2023)

The plant is commonly called Be-still tree or lucky nut. Its genus name *Cascabela* came from the Spanish word meaning small bell. (Plants.ces.ncsu.edu, 2023) and the name *thevetia* is a latin name in honour of Andre Thevet (1516-1590) a franciscan priest who was a French man that explore Brazil and Napoleons Hat.(en.m.wikipedia.org; and www.missouribotanicalgarden.org. 2021).

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C.thevetia are widely cultivated as ornamental plants and it is toxic when ingested by vertebrate due to cardiac glycosides they contain. (Shannon and Paul, 1996; Sttenkamp, 2005.)

The seed is said to be poisonous when ingested, research has shown the *C. thevetia* plant to be toxic to most vertebrates due to cardiac glycosides they contain. (Shannon and Paul, 1996 & Steenkamp, 2005).

The world supply of vegetable oil is currently in excess of 100 million metric tons. The demand is on the increase due to incrseasing population and increasing demand on vegetable oil for non- food uses likes production of biodiesel, lubricant cosmetics e.t.c

However, only about 12 of the 500,000 known plant species are currently exploited on a commercial rate for production of oil to meet the world rising demand. (www.sciencedirect.com. 2016).

Hence the search for more sources of non-edible oil is required to reduce the demand and dependability on edible oil by both cosmetics and industrial organization.

Despite the poisonous nature of the plant, it can still be useful. Records has it that, the bark is use as strong anti-periodic and febrifuge, it is also use in treatment of malaria fever and snake bite. Juice from the bark is use externally to treat sores. (http://wiki.medicinalplantsuses .com, 2016) *C. thevetia* can be a very potential source of oil for industrial uses

Phytochemicals originate from plants, they help plants to fight or resist bacterial, fungi and virus infections. Phytochemicals are natural bioactive compounds found in plants that are responsible for the taste, aroma and colour of foods and plants. Plants, fruits, vegetables, flowers, leaves and roots contain phytochemicals which serve as defence mechanism against diseases, or more accurately protect plants against diseases (Ayo, 2010).

Microganism: These are also called microbe which are usually microscopic size in nature. They can only be seen with the aid of a microscope; microorganism can exist in colony of cells. (www.en.m.wikipedia.org. 2022).

Microorganism despite the fact that they cause disease conditions to human, they are also important to human in many ways. They are use in food fermentation, sewage treatment, bioactive fuel production and also enzymes. These microbes are also use as model, biological warfare and bioterrorism and are vital component of fertile soil. (Schopf, 2017).

The microorganism use for this study are *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Rhizopus stolonifer* and *Candida albican*

2. Methodology

Method for phytochemical: Qualitative phytochemical method was carried out on the sample using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harbone (1973).

Method for antimicrobial: The modified agar well diffusion method was employed to determine the antimicrobial activities for n-hexane extract of *C. thevetia* seed oil. Different concentration of the extract 500 µg/m, 250 µg/m, 125 µg/m, and 6.25 µg/m were prepared, this is to aid in knowing the least concentration that will be required for the culture to grow. (Collins *et. al.,* 1995). 0.1ml of standardized 24hours old culture of the tested organisms in nutrient broth was spread unto sterile prepared nutrient agar plates and allowed to set. With the aid of a sterile 9 mm cork borer, hole was bored on the plates. 0.5 ml of each concentrations of the extracts were dispensed into the hole and left to stand for 15minutes. The prepared standard solution of gentamicin 0.1 ml was also dispensed into the hole. These were then incubated at 37^{o}c for 24 hours. At the end of the incubation period the zones of inhibitions were measured using scientific ruler and the values recorded. (Junaid *et. al.,* 2006).

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was determined by measuring about 5mls of nutrient broth into empty sterilized tubes. 1 ml of the different concentrations was added and incubated for 24 hours at 37^{o}c. The tube having the least concentration of extract that shows least growth was determined as the MIC while the tubes with the least growth were poured on a nutrient agar plate and incubated for 24 hours at 37^{o}c, the plate with the least concentration of extract that showed no growth at the concentration was reported as the MBC (Junaid *et.al.,* 2006)

3. Results

Table 1 Qualitative and Quantitative Phytochemical screening of *C. thevetia* seed oil

Phytochemicals	Qualitative	Quantitative
Alkaloids	+	0.062 ± 0.001
Flavonoids	+++	3.123 ± 0.005
Glycosides	+	0.163 ± 0.010
Phytates	+++	4.530 ± 0.014
Saponins	++	2.920 ± 0.010
Tannins	+++	3.130 ± 0.014

Key: + = slightly present; ++ = moderately present; +++ = Highly present.

Results for quantitative analysis were performed in triplicate and expressed as mean ± SD

Table 2 Antimicrobial analysis of *C. thevetia* seed oil in mm

Species of Organism	Zone of inhibition C.thevetia oil	Zone of inhibition Gentamicin (Bac)	Zone of inhibition Fluconazole (Fun)
Staphylococcus aureus	15.33	24	-
Pseudomonas aeruginosa	12.00	25	-
Salmonella typhi	16.67	23	-
Escherichia coli	16.00	20	-
Rhizopus stolonifer	12.00	-	22
Candida albicans	11.00	-	22

Results for antimicrobial activity were performed in triplicate and expressed as mean ± SD.

Table 3 Minimum inhibitory concentration (MIC) mg/mL of *C. thevetia* seed oil

Species of Organism	50 mg/ml	25 mg/ml	12.50 mg/ml	6.25 mg/ml	3.125 mg/ml
Staphylococcus aureus	N	N	A*	B	B*
Pseudomonas aeruginosa	N	N	A*	B	B*
Salmonella typhi	N	N	A*	B	B*
Escherichia coli	N	N	A*	B	B*
Rhizopus stolonifer	N	N	N	A*	B
Candida albicans	N	N	N	A*	B

Key: N= No growth, A*= MIC, B= Light Turbidity, B*= Moderate Turbidity

The MIC value for bacterial was obtained at 12.5 mg/ml while for the fungi the MIC was 6.25 mg/ml

Table 4 Minimum Bactericidal/Fungicidal Concentration of *C. Thevetia* Seed Oil

Species of Organism	50 mg/ml	25 mg/ml	12.50 mg/ml	6.25 mg/ml	3.125 mg/ml
Staphylococcus aureus	N	A*	B	B*	C
Pseudomonas aeruginosa	A*	B	B*	C	D
Salmonella typhi	A*	B	B*	C	D
Escherichia coli	A*	B	B*	C	D
Rhizopus stolonifer	N	A*	B	B*	C
Candida albican	N	A*	B	B*	C

Key: N= No growth, A*= MBC/MFC, B= Scanty growth of colonies, B*= Moderate growth of colonies, C= Heavy growth of colonies, D= Very heavy growth of colonies

The MBC value was obtained at 50 mg/ml for *Pseudomonas aeruginosa, Salmonella typhi* and *Escherichia coli. Staphylococcus aureus* have MBC value of 25mg/ml which is the same value of MFC obtained for *Rhizopus stolonifer* and *Candida albican.*

4. Discussion

Table 1 above showed the qualitative and quantitative analysis of the six phytochemicals investigated to be present with Alkaloids and Glycosides to be slightly present. Saponins moderately present while Phytates, Flavonoids and Tannins to be highly present.

The quantitative results showed 0.062 ± 0.001; 3.123 ± 0.005; 0.163 ± 0.010; 4.530 ± 0.01; 2.920 ± 0.010 and 3.140 ± 0.014 mg/g for Alkaloids, Flavonoids, Glycosides, Phytates, Saponins and Tannins respectively. Plants are usually of medicinal and industrial importance due to the phytochemicals present in them. Flavonoids and Tannins have been reported to show antimicrobial activity. (Yebpella *et al.,* 2011 and Hammuel *et al.,* 2011). Abhilasha and Kuntal (2013) reported that plants having phytochemicals like Alkaloids, triterpenes, flavonoids e.t.c have therapeutic properties (anti-inflammatory, anti-microbial) which makes them valuable medicinally.

Table 2 shows the antimicrobial activity of *C. thevetia* seed oil was carried out using agar well diffusion method against *Staphylococcus aureus* a gram-positive bacterial, *Pseudomonas aeruginosa, Salmonella typhi* and *Escherichia coli* gram-negative bacterial with fungi *Rhizopus* and *Candida albicans.* Gentamicin and fluconazole were used as standard. *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi* and *Escherichia coli* shows zone of inhibition of 15.33, 12.00, 16.67 and 16.00 mm respectively. The fungi showed zone of inhibition of 22 mm for both *Rhizopus stolonifer* and *Candida albican.*

Table 3 shows the MIC value for bacterial obtained at 12.5 mg/ml while for the fungi the MIC was 6.25 mg/ml while the MBC was obtained at 50 mg/ml for *Pseudomonas aeruginosa, Salmonella typhi* and *Escherichia coli. Staphylococcus aureus* have MBC value of 25 mg/ml which is the same value for MFC obtained for *Rhizopus stolonifer* and *Candida albican,* this is similar to Hammuel *et al., (*2011) report for methanolic *Thevetia peruvana* oil extract with MIC value of 12.5 mg/ml and MBC of 50 mg/ml, while the chloroform extract to have MIC of 6.25 mg/ml and the MBC of 25 mg/ml on the microorganisms they inhibited.

Table 4 shows the minimum bactericidal/fungicidal concentration of *C. thevetia* seed oil.

Pseudomonas aeruginosa, Salmonella typhi and *Escherichia coli* shows minimum bactericidal concentration at 50 mg/ml, *Staphylococcus aureus* have a minimum bactericidal concerntration value of 25 mg/ml which is the same value for minimum fungicidal concentration obtained for *Rhizopus stolonifer* and *Candida albican.*

The antimicrobial activities of *C. thevetia* oil show that the presence of bioactive compound(s) can serve as antimicrobial agent.

5. Conclusion

The oil has showed great phytochemical properties and antimicrobial activities hence it can be use in the pharmaceutical industries for production of novel drugs and skin creams for treatment of infections.

The plant is of promising economical value when invested into and duly utilized.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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