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Phytochemicals And Antimicrobial Potential Of Citrullus Vulgaris Seed And Pod Extracts

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Abstract: ***Medicinal plants play a key role in the human health care system. Herbal medicines are in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects. Fruits and vegetable have been recognised as natural source of various bioactive compounds that can inhibit microbial growth or cure aliment. Citrullus vulgaris of the family cucurbitaceous is commonly known as watermelon possess antimicrobial activity due to its nutritive values. Unlike conventional drugs (Antibiotics), C. vulgaris plant has little or no side effects and is not expensive. The study was carried out to determine the phytochemicals and the antimicrobial potential of C. vulgaris. C. vulgaris seeds and pod were extracted by maceration with warm water and by soxhlet extraction using n-hexane and ethyl acetate, the filtrates obtained were concentrated and kept at low temperature until used. Presence of alkaloids, diterpenes, glycosides, flavonoids, lactones saponins, steroids, tannins and Triterpenes were determined using standard methods. Antimicrobial activity of the extracts against different species of bacterial and fungi was evaluated. Phytochemical screening revealed the presence of steroids, Triterpenes, alkaloids glycosides among others. The extracts were found to exhibit potency on the strains of bacteria and fungi used. The antimicrobial potential of the extracts could be attributed to the presence of some phytochemicals.***

I. INTRODUCTION

Medicinal plants play a key role in the human health care system. About 80% of the world population rely on traditional medicine which is predominantly based on plant medicine. Plant medicine (Phyto-medicine) has been used in healthcare delivery in many part of Africa and the rest of the world (Elujoba *et al*., 2005). Effective health cannot be achieved in Africa, unless orthodox medicines is complemented with traditional medicine (Elupoba *et al*., 2005). Fruits and vegetable have been recogni*se*d as natural source of various bioactive compounds that can inhibit microbial growth or cure aliment (Pennington and Fisher, 2010) which could be attributed to the presence of phytoconstituents such as flavonoids, tannins, vitamins C and E, Phenols, glycosides present in fruits and vegetables (Gonz' alez- Aguilar, *et al*., 2008). *Citrullus vulgaris* of the family cucurbitaceous is commonly known as watermelon. The ripe fruits are edible. Its nutritive values are also useful to the human health. It also reported having analgesic and anti-inflammatory activity of roots and leaves, antimicrobial activity, antioxidant, anti-diabetic activity (Dong *et al*., 2011; Loly *et al*., 2011; Gill *et al*., 2010; Jiyun *et al*., 2011). *Citrullus vulgaris* seeds have both nutritional and cosmetic importance, the seed contains vitamins B2, Minerals, riboflavin, fat, carbohydrates and proteins. *Citrullus vulgaris* may have antibacterial, antifungal and antihypertensive effects among other health benefits (Loly, *et al*., 2011). Hassan *et al*., 2011 reported a number of trado-medical uses of different part of watermelon including diuretic, diabetes, pectoral, tonic, vermifuge and in the treatment of nocturnal enuresis. Although most studies on medicinal use of this plant were using leaves, fruits, roots or

flowers, it is anticipated that the seeds could also be effective as the other parts of the plant due to large number of chemical compounds present in the seeds or seed coats, including alkaloids, Lectins, and phenolic compounds such as lactones, Tannins, and Flavonoids (Borchardt *et al*., 2008). Although these were believed to be for plant's protection, but have also been reported to play important role as antimicrobial (Braide *et al* 2012).This research was designed with view to evaluate the antimicrobial efficacy of the seed and pod extracts against some selected bacterial and fungal species.

II. MATERIALS AND METHODS

PLANT MATERIAL

Fresh *Citrullus vulgaris* seeds were purchased from Ipata Market, Ilorin, Kwara State, Nigeria, in May, 2018. The authentication of the plant was done at the herbarium unit of the Plant Biology Department of the University of Ilorin, Ilorin where a voucher number of UILH/001/1208.

MICROORGANISMS

Esherichia coli, *Klebsiella Sp*., *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*. Were obtained from Microbiology Unit of Science Laboratory Department, Kwara State Polytechnic, Ilorin, Kwara State, Nigeria.

CHEMICALS AND REAGENTS

All chemicals used were of analytical grade and were obtained from Tianjin Kermel Chemical Reagent Co. Ltd. Republic of china.

III. METHODS

A. PREPARATION OF EXTRACTS

Citrullus vulgaris seeds were removed from the pod and were grounded to powder using a blender (Master Chef blender, model No: MCBL6CH) and the pods were also grounded to powder using a blender (Mikachi blender, Model No: MK – 999).

Warm extraction by maceration was carried out on the powdered pod using warm water as described by (Tiwari *et al*., 2011) as follows: The powdered pod (10g) was weighed and extracted in 50ml warm water in a beaker. The mixture was kept for 10 minutes and then filtered with whattman filter paper into a clean beaker.

Soxhlet extraction of the powdered pod and powdered seed carried out using two solvents n-Hexane and Ethyl acetate. The powdered seed and pod (200 g and 250 g respectively) were separately subjected to solvent extraction in Soxhlet apparatus with 750ml of n-Hexane and 750 ml ethyl acetate at different time. The extracts obtained were concentrated by distillation to remove the solvent and the residue were weighed and stored in air-tight container until used (Varghese *et al*., 2013).

a. DILUTION OF EXTRACTS OF ANTIMICROBIAL

Each of the extracts (0.10 g) were re-dissolved in 100 ml of each of the corresponding solvents. The dilutions were mixed together and kept in air tight container for antimicrobial activity.

b. QUALITATIVE ANALYSIS OF SECONDARY PHYTOCHEMICALS

Extracts of all plant parts (seeds and pod separately) were evaluated for preliminary screening of secondary phytochemicals, alkaloids and saponins were determined following the method described by Harbone (1973), flavonoids, Diterpenes, Tannins, Steroids and Terpenoids were determined using the procedure describe by Odebiyi and Sofowora (1978). Glycosides, were determined using the procedure described by (Trease and Evans 1985). All the determination were done with slight modification.

c. PREPARATION AND STERILIZATION OF MEDIA

Mueller Hinton agar was prepared by dissolving 20 g of the agar powder in 500 ml of distilled water. Heat was applied with continued stirring until the solution dissolved. The pH was adjusted to 7.4 and the agar steri*ise*d at 121°C for 15 minutes using Autoclave. Potato dextrose agar was prepared by dissolving 20 g of the agar powder in 500ml of distilled water. The pH was adjusted to 7.4 and the agar steri*ise*d at 121°C for 15 minutes using Autoclave.

d. PREPARATION OF DISC INFUSION

Paper perforator machine was used to perforate whatmann filter paper into small s*ise*s, the perforated filter papers were soaked in the extracts. It was then removed and allowed to dry (Lalitha, 2014).

B. ANTIMICROBIAL ASSAY

For antibacterial assay, sterilised, molten Mueller Hinton agar (20 ml) was poured into a set of sterilised petri dishes under aseptic condition and was allowed solidify then each plate was inoculated with pure cultures of the bacterial test organisms and the extract infused discs were placed on the culture medium and inoculated at 37°C for 24 hours. Plates were subsequently observed for zones of inhibition. For antifungal assay, sterilised, molten Potato dextrose agar (20 ml) was poured into a set of sterilised petri dishes under aseptic condition and was allowed to solidify then each plate was inoculated with pure cultures of the fungal species and the extract – infused discs were placed on the culture medium and inoculated at 37°C for up to 48 hours. Plates were subsequently observed for zones of inhibition.

IV. RESULTS

SECONDARY METABOLITES DETECTED IN CITRULLUS VULGARIS SEED AND POD EXTRACTS

Secondary metabolites detected in the extracts are shown in Table 1. These metabolites include Alkaloids, Flavonoids, Saponins, Glycosides, Tannins, Steroids, Diterpenes, Lactones and Triterpenes,

Phytochemicals	n-Hexane Extract		Ethylacetate Extracts		Aqueous extract
	Pod	Seed	Pod	Seed	Pod
Steroids	+	+	-	-	-
Triterpenes	+	+	-	-	-
Alkaloids	+	+	+	+	+
Glycosides	+	+	+	+	+
Diterpenes	-	+	+	+	+
Tannins	+	-	+	-	+
Lactones	-	-	+	+	-
Flavonoids	-	-	+	-	+
Saponins	-	+	-	-	+

+ = detected; - = not detected

Table 1: Secondary Metabolites detected Citrullus Vulgaris Seed and Pod Extracts

ANTIBACTERIAL POTENTIALS OF DILUTED AND UNDILUTED SEED AND POD N-HEXANE EXTRACTS OF CITRULLUS VULGARIS ON SELECTED BACTERIAL STRAINS

Antimicrobial potential of Citrullus vulgaris Diluted and Undiluted Seed and Pod Extracts are shown in Table 2. Potency was observed on the bacterial strain of *pseudomonas aeruginosa* that were treated with n-Hexane diluted seed and pod extracts. Potency was also observed on the bacterial strain of *Escherichia coli* treated with n-Hexane undiluted seed extracts, however potency was not observed in other bacterial strains investigated.

Bacterial	Diluted seed	Diluted pod	Undiluted seed	Undiluted pod
E. coli	-	-	+	-
S. aureus	-	-	-	-
P. aeruginosa	+	+	-	-
Klebsilla Sp.	-	-	-	-

+ = potent; - = Not potent

Table 2: Antibacterial potentials of Diluted and Undiluted Seed and Pod n-Hexane Extracts of Citrullus vulgaris on Selected Bacterial Strains

ANTIFUNGAL POTENTIALS OF DILUTED AND UNDILUTED SEED AND POD N-HEXANE EXTRACTS OF *CITRULLUS VULGARIS* ON SELECTED FUNGAL STRAINS

Antifungal potentials of Diluted and Undiluted Seed and Pod n-Hexane Extracts of *Citrullus vulgaris* are shown in Table 3. Potency was not observed in fungal strains treated with diluted seed pod n-hexane extracts of *Citrullus vulgaris*. Potency was observed on the fungal strain of *Aspergillus niger* treated with undiluted seed and pod n-hexane extracts, however potency was not observed in fungal strain of *Candida albicans*.

Fungal	Diluted seed	Diluted pod	Undiluted seed	Undiluted pod
C. albicans	-	-	-	-
A. niger	-	-	+	+

+ = Potent; - = Not Potent

Table 3: Antifungal Effect of n-Hexane Extracts of Citrullus vulgaris Seed and Pod against selected Fungal Strains

ANTIBACTERIAL POTENTIALS OF DILUTED AND UNDILUTED SEED AND POD ETHYL ACETATE EXTRACTS OF *CITRULLUS VULGARIS* ON SELECTED BACTERIAL STRAINS

Antibacterial potentials of diluted and undiluted seed and pod ethyl acetate extracts of *Citrullus vulgaris* on selected bacterial Strains are shown in Table 5. Potency was not observed in bacterial strains treated with diluted and undiluted seeds ethyl acetate extract of *C. vulgaris*. However, potency were observed on the bacterial Strains of *S. aureus, P. aeruginosa* and *Klebsiella Sp* that were treated with diluted pod. Potency was also observed in *Klebsiella Sp* treated with undiluted pod.

Bacterial	Diluted seed	Diluted pod	Undiluted seed	Undiluted pod
E. coli	-	-	-	-
S. aureus	-	+	-	-
P. aeruginosa	-	+	-	-
***Klebsilla* Sp.**	-	+	-	+

+ = Potent; - = Not Potent

Table 4: Antibacterial Potentials of Diluted and Undiluted Seed and Pod Ethyl acetate Extracts of Citrullus vulgaris on Selected Bacterial Strains

ANTIFUNGAL POTENTIALS OF DILUTED AND UNDILUTED SEED AND POD ETHYL ACETATE EXTRACTS OF *CITRULLUS VULGARIS* ON SELECTED BACTERIAL STRAINS

Potency was not observed in all the fungal strains treated with undiluted seed, undiluted pod and diluted seed extract of Ethyl acetate were not potent. However, potency was observed in *Aspergillus niger* treated with diluted pod Extract of Ethyl acetate.

Fungal	Diluted seed	Diluted pod	Undiluted seed	Undiluted pod
C. albicans	-	-	-	-
A. niger	-	+	-	-

+ = Potent; - = Not Potent

Table 5: Antifungal Potentials of Diluted and Undiluted Seed and Pod Ethyl acetate Extracts of Citrullus vulgaris on Selected Bacterial Strains

V. DISCUSSION

Plants have been used as drugs and therapies for various ailments. *Citrullus vulgaris* seeds and pods were found to contain phytochemicals including Alkaloids, Tannins, Diterpenes, Glycosides, Lactones, Flavonoids, Saponins, Steroids, Triterpenes. the biological activities shown by plants are due to the presence of phytochemicals. The role of these phytochemicals as antimicrobial has been reported by many researchers (Okorondu *et al*., 2010; Hassan *et al*., 2011 and Saxena *et al*., 2013). The presence of these phytochemical in *C. vulgaris* seeds was also reported by many authors (Braide *et al.*, 2012; Nwankwo *et al*., 2014 and Godwin *et al*., 2015). Plants containing alkaloids have mechanism by which they resist pests including microorganisms (Izah *et al*., 2016). Typically, alkaloids and their synthetic derivatives are effective for the treatment of antibactericidal orientated diseases (Osuntokun and Oluwafoise, 2015). Flavonnoids have several medicinal properties including antioxidant and antimicrobial, the presence of saponins in plant ai an indication that such plant could be used as expectorant, cough suppressant (Osuntokun and Oluwafoise, 2015). Several researchers have reported the medical importance of tannins for the treatment of wounds and burns (Kigigha *et al*., 2015; Osuntokun and Oluwafoise, 2015 and Izah *et al*., 2016). The presence of Saponins in this study was in contrast to the works reported by Nwankwo *et al.* (2014), Adelani–Akande *et al*. (2015) and Godwin *et al*. (2015) but corresponds to that of Hassan *et al*. (2011) and Braide *et al*. (2012). The disparities observed might be attributed to difference in solvent used for extraction, and/or geographical location as it can affect plant's active constituents, which may include many factors like soil, climate and propagation method (Nwankwo et al., 2014). Presence of these phytochemicals in the extracts was a clear indication of antimicrobial potentials of *Citrullus vulgaris* seeds.

Antibacterial activity of the seed and pod extract of *Citrullus vulgaris* showed that all the organisms tested were susceptible. For bacterial strains, the Ethyl acetate extracts of diluted pod, and undiluted pod, the n-Hexane extracts of diluted seed diluted pod and undiluted seed were seen to exhibit potency on *Pseudomonas aeruginosa, Klebsiella Sp, Staphylococcus aureus and Escherichia coli.* Ethylacetate extracts of diluted pod exhibit more potency on the bacterial strains of *S. aureus, P. aeruginosa, and Klebsiella Sp. investigated*. Comparatively, different researchers have reported contradictory observations in this regards (Braide *et al.*, 2015; Egbuonu, 2015). This contradiction mighty be a function of methodological differences, Strains variability, and/or nature of solvent used for extraction. Susceptibility of *S. aureus* and *E. coli* observed in this study is in agreement with that of Godwin, *et al*. (2015) who made similar observations. In a study by Adunola *et al*. (2015) susceptibility of *S. aureus* was attributed to the presence of saponins, while the work reported by Bello *et al*. (2016) supported the view that other phytochemical beside saponins are active against Gram positive bacterial also.

Antifungal activity of the seed and pod extracts showed that not all the organisms tested were susceptible. Ehtyl acetate extract of diluted pod, n-Hexane extract of undiluted seed and undiluted pod showed potency on *Aspergillus niger*, suggesting the potency of these extracts of *Citrullus vulgaris* against *Aspergillus niger* and possible diseases caused by *A. niger*. *Candida albicans* was not susceptible to any extracts of *Citrullus vulgaris*. The impotency of the extracts *Candida albicans* in this study contradicts the works reported by (Egbuonu, 2015). This might be as a result of the solvent used for extraction.

VI. CONCLUSION

It can be concluded that the antimicrobial potentials of seed and pod extracts of *Citrullus vulgaris* against selected bacteria and fungi might be due to the presence of some phytochemicals. The activity observed is a clear indication of therapeutically property possessed by seeds and pods of the plants. Therefore, the seeds and pods of *Citrullus vulgaris* could be a good source of antimicrobial agents and thus, could be consumed by human.

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