
ANTI-FUNGAL ACTIVITIES OF *ALLIUM SATIVUM* AQUEOUS EXTRACT ON IRREFUTABLE DERMATOPHYTES IN BAUCHI STATE, NIGERIA

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ABSTRACT

*Dermatophytic contagion is a communal infection that creates public health menace. The study was targeted at determining the anti-fungal actions of aqueous garlic extracts on dermatophyte so as to avail natural and cost effective remedy to victims of dermatophytic contagion. The actions of aqueous extracts of garlic (*Allium sativum*) on dermatophytes were reconnoitered on isolates of these disease causing fungi secluded from one hundred and thirty three patients attending Infectious Disease Hospital Bayara in Bauchi state. Out of the 133 isolates cultured, growths identified to be fungi were observed in only 60 petri dishes. The pattern of the organisms' response to aqueous extraction was ascertained by the use of disk diffusion technique. The outcomes were matched with the achievements of a customary antifungal medication called Griseofulvin. The isolates encompassed; *Trichophyton violaceum*, *Trichophyton mentagrophytes*, *Trichophyton tonsurans* and *Trichophyton rubrum*. The effect of the antifungal operations of aqueous garlic extract disclosed high butwide-ranging intensities of antifungal efficacy on the dissimilar species of the dermatophytes at five diverse intensification or application of; 400 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, and 25mg/ml. *Trichophyton violaceum* had the maximum susceptibility trailed by *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *T. tonsurans* respectively. Distance regions of inhibition displayed by the extracted concentrate of garlic in opposition to fungal species fluctuated between the minimum mark of 5.50 mm and maximum mark of 36.67 mm. Aqueous Garlic extract showed very good potential as an antifungal agent.*

Key words: Antifungal/Counter-fungal action, Garlic (*Allium Sativum*) Aqueous concentrate, Dermatophytes,

INTRODUCTION

The usage of vegetation as a basis of medication to treat communicable disease started as far back as pre-historic era. Almost every culture and nation have used herbal medicines to remedy disease (Lino and Deogracious, 2006; Sofowora, 2008). Dermatophytes exemplify the predominant kind of fungi that initiates infection of the skin, hair and nails (Ameen, 2010). Because of the associated resistance, deficient efficacy and side effects resulting from the use of some of the existing drugs, substantial consideration has been given to plant concentrates for the treatment of fungal contagions. *Allium sativum* has a place in the lily family; *Liliaceae*. It is generally known; garlic and is mostly available in the tropics. It's physiognomic odour and taste is traced to the aromatic Sulphur-centered composites it contains.

Garlic (*Allium sativum*) has been widely recognized to have inhibitory actions on a number of pathogenic fungi and viruses, bacteria (Sofowora, 2008). This study checked out the phytochemical breakdown of Garlic (*Allium sativum*), isolate, identify dermatophytic fungi from the Bayara study population and determine the antifungal attainment of Garlic (*Allium sativum*) juices on dermatophytic fungi segregates.

MATERIALS AND METHODS

This research was conducted in the microbiology laboratory of Abubakar Tafawa Balewa University, Bauchi. Ethical clearance was obtained from the institution and ministry of health Bauchi, Bauchi state.

STUDY POPULATION

The study population constitutes patients attending infectious disease hospital Bayara, Bauchi. The predominant occupations of the people are farming and livestock rearing. The hospital is situated in by the road side along Bauchi Tafawa Balewa road within Bauchi local government of Bauchi state. The city has a population of 316,173 (2004). Geographically, Bauchi State inhabits an aggregate land space of 49,119 km² and is situated between latitudes 9° 3' and 12° 3' north and longitudes 8° 50' and 11° east. Bordering states at the north are; Kano and Jigawa, Plateau and Taraba to the south, Kaduna to the west, Yobe and Gombe to the east (Bauchi State Government BASG, 2018).



Figure 1: Map of Bauchi State highlighting Bauchi Local Government, the study area.

The model size was decided by means of Thrustfield formulation and 9.6% prevalence from previous studies;

$$N = \frac{Z^2 P (1-P)}{d^2}$$
$$N = \frac{(1.96)^2 \times 0.096(1-0.096)}{(0.05)^2}$$
$$N = \frac{3.846 \times 0.0867}{0.0025}$$

$$N = 133$$

(Ezike *et al.*, 2004, Thrustfield, 2007).

An aggregate of 133 samples obtained from patients who presented observable irrefutable signs of dermatophytic infection such as scaling, erythema, pruritus, crusting, follicular inflammation or circinate lesions among others. Fresh garlic bulbs were purchased from Yelwa market and identified in the herbarium of Abubakar Tafawa Balewa University Bauchi State. Garlic bulbs were de-husked, washed with sterilized distilled water, subjected to air dry and pounded into powder. The procedure of extraction was according to the technique designated by Doherty *et al* (2010). The concentrate was squeezed out from 100 g of the powdered sample using water as solvent. Each 100 g powdered sample was saturated in water for 24 hours over the night; then sieved with muslin material. The juice was gathered in a round bottom bottle, concentrated by means of a rotary evaporator and subjected to oven drying at 40 °C. The extraction was vetted for the manifestation of bioactive chemicals such as; anthroquinones, alkaloids, tannins, saponins, Steroids, Glycosides, Flavonoids and Terpenoids (Soforowa, 1982; Segelma *et al.*, 1971; Trease and Evans, 2002; Wall *et al.*, 1954). The dehydrated extract was refreshed by liquefying 0.4g of aqueous extract in 1ml of dimethyl sulphur oxide (DMSO) and was expressed as 400 mg/ml preparation. By further

dilution of suitable DMSO volumes, the preparations of 50 mg/ml, 100mg/ml and 200mg/ml were prepared. DMSO was chosen as the liquefying solvent because it dissolves both polar and non-polar compounds unlike water which dissolves only polar substances. Griseofulvin tablets were crushed into powder, 0.4g of its powder was dissolved in 1ml of DMSO resulting in 400mg/ml concentration, 0.2g of Griseofulvin powder was dissolved in 1ml of DMSO resulting in 200mg/ml concentration, 0.1g of Griseofulvin powder was dissolved in 1ml of DMSO resulting in 100mg/ml concentration and 0.05g of Griseofulvin powder was dissolved in 1ml of DMSO resulting in 50mg/ml concentration.

Skin Scraping was done, by means of sterilized surgical blade and positioned in hygienic, well-labeled envelope as specified by Cheesebrough (2002) then conveyed to the microbiology laboratory of Abubakar Tafawa Balewa University Bauchi. The preparation of Potatoes Dextrose Agar (PDA) medium was in accordance to the manufacturer's stipulations. Thereafter, the medium was dispensed into sterilized Petri dishes and allowance was made for solidification to take place. Inoculation of fungi (scales) obtained from each participating patient in the study was made into the PDA dishes and subjected to incubation for four to seven days at room temperature (25°C to 35°C). The culture media were observed for dermatophytes' growth and colonies were Sub-cultured to acquire distinctive growth of dermatophyte (Collins *et al.*, 2004).

Confirmatory cultures were observed macroscopically for the produced pigmentation, colour, colony texture and topography as (Collins *et al.*, 2004). Positive cultures were stained with lacto phenol cotton blue (LPCB) and microscopically viewed at 10x and 40x magnification for conidia (Collins *et al.*, 2004). A spotless glass slide was positioned on the work bench and LPCB solution was dropped in small amount at the center of the slide. About 1-2 mm section of a fungal colony was detached from the margin with a suitable, sterile stick and released on the LPCB solution. The section of a fungal colony was mildly teased using two sticks awaiting separation. Thereafter, there was a gentle placement of coverslip on it and a carefully examination of the slide under 10X (low power) and 40X (high power) microscope's objectives for the distinguishing arrangement and shape of the hyphae and spores (Collins *et al.*, 2004). Kirby Bauer diffusion disk method was employed in the determination of the counter-dermatophytic actions of aqueous garlic concentrates (Reynolds, 2019). The filtering discs were infused with extracts of garlic at diverse concentrations; 25 g/ml, 50 g/ml, 100 g/ml, 200 g/ml, and 400 g/ml. with the aid of sterilized tongs, the infused discs were positioned on the surface of Mueller- Hinton Agar dish that contains a suspension of 0.5 McFarland (1.5×10^8 cells) turbidity from a 4 days old culture of segregates and the dishes subjected to incubation at ambient temperature. The efficacy of the extract was revealed by the incidence of growth-restrained regions. These regions of restrained better known as Zone of inhibition (ZOIs) become clearly visible areas around the disc diffusing counter-fungal substances. The diametric coverage of ZOI were ascertained in millimeter using a ruler (Soforowa, 1982) and articulated as mean \pm Standard Error of Mean (SEM).

ETHICAL CONSIDERATION: Ethical clearance with reference MOH/GEN/S/1409/1 was obtained from the Ministry of Education, Bauchi state, Nigeria.

RESULTS AND DISCUSSION

Results of the phytochemical study of *Allium sativum* (Garlic) aqueous concentrate showed the presence of saponins, terpenoids, and flavonoids. Mungole *et al.*, (2010) reported that these phytochemical constituents inhibit fungal growth. Saponins observed in the plant are known to possess expectorant activity and impede fungi growth (Banso, 2009). The counter-

fungal accomplishments of flavonoids have been testified by a good number of researchers (Aliero *et al.*, 2008; Yasmin *et al.*, 2008).

As natural substitutes for artificial compounds, garlic's phytochemical derivatives have fascinated great interest. Natural products make available hints to the manufacture of new structural brands of antifungal substances that are comparatively harmless to humanity. The manifestation of organically active compounds; flavonoids, terpenoids and saponins in the aqueous garlic concentrates could be connected to its antifungal effects (Dubey and Jagannadham, 2003). Probably, these molecules are the major counter-fungal agents in the juice extracts of *Allium sativum*. This gained the agreement of Toryali *et al.*, (2018) who stated that; the phytochemical screening of garlic extract showed the existence of volatile oil, saponin, amino acids, terpenoid, flavonoids, cardiac glycosides and proteins. Flavonoids are polyphenolic fragments that are soluble in water and possess antioxidant undertakings alongside great health boosting effects and antimicrobial possessions (Trease and Evans, 2002).

OCCURRENCE OF DERMATOPHYTES AMONG STUDY POPULATION

The number of samples collected can be seen on Table I. Clinical manifestations found, shows that the greatest and popular irrefutable abrasions of dermatophytosis were; *Tinea corporis* then *Tinea unguium*, followed by *Tinea capitis*. The dermatophytes isolated were *Trichophyton mentagrophytes*, *Trichophyton tonsurans*, *Trichophyton violaceum* and *Trichophyton rubrum*. *Trichophyton mentagrophytes* was found to be the most occurring with 30 cases (50%) followed by *Trichophyton violaceum* with 14 cases (23.3%). This is attributable to the patients' contact with soil and animals that live at home. This result is similar to Jha *et al.* (2012) who reported that the highest popular genus was *Trichophyton*, trailed by *Epidermophyton*. This also agrees with Kannan *et al.* (2006), who described that *Trichophyton rubrum* at (73%) was the chief popular isolate, trailed by *Trichophyton mentagrophytes* at (17%), *Trichophyton violaceum* at (12%), and finally *Epidermophyton floccosum* at (8%).

Table I: Distribution of dermatophytes isolated from the study

Dermatophytes	No. of Isolates (n = 60)	% Occurrence
<i>Trichophyton mentagrophyte</i>	30	50
<i>Trichophyton rubrum</i>	10	16.7
<i>Trichophyton Violaseum</i>	14	23.3
<i>Trichophyton tonsurans</i>	6	10

ANTIFUNGAL ACTIVITY OF ALLIUM SATIVUM (GARLIC) AQUEOUS EXTRACT

In Aqueous solution, differences in the mean zone of inhibition of *T. mentagrophyte* occurring between the control concentration (21.5±9.2), 50 mg/ml (6.5±2.1) and 100 mg/ml (11.0±1.4) were not significantly different from each other (Table II). The least mean inhibition zone was at 25 mg/ml (0.0±0.0) concentration and the highest at 400mg/ml (33.0±4.2). For the *T. rubrum* organism, the mean zone of inhibition for the control (8.0±0.0) was not significantly different from 50mg/ml (7.5±0.7). The mean zone of inhibition at 200mg/ml (24.5±0.7) and 400mg/ml (29.5±6.4) were also not significantly different from each other. The least zone of inhibition was at control (3.0±4.2). The concentration at 400mg/ml had the maximum mean of inhibition zone. The *T. tonsurans* organism had its

highest mean zone of inhibition at 400 mg/ml (27.5±2.1) which was not significantly different from the mean at 200 mg/ml (24.0±0.0). The *T. violaceum* organism had its maximum mean of inhibition zone at 400 mg/ml (31.0±8.5) which was significantly higher than the mean zone of inhibition at all other concentrations; 25 mg/ml (0.0±0.0), 50mg/ml (6.0 ± 0.0), 100mg/ml (10.0 ± 0.0), 200mg/ml (24.0 ± 0.0) and the control (15.0 ± 4.2) (Table II). Garlic (*Allium sativum*) in this current study has demonstrated an ability to impede fungi growth at the time of testing. The outcome has evidently specified that garlic aqueous juice-extract has high inhibitive action.

The inhibitory effect gain increase with higher concentration of the juice-extract. Anti-dermatophytic activity of garlic aqueous extract on four dermatophytes fungi isolated from 60 patients was experiential in this very study and validates the work by Aala *et al.* (2010) who stated the action of garlic against irrefutable isolates of dermatophytes including; *E. floccosum*, *T. rubrum*, *M. canis*, *T. verrucosum*, and *T. mentagrophytes*. The result is also similar to Ami and Kapadnis (2005) who examined the counter-fungal activity of garlic in opposition to *T. mentagrophytes* and *T. rubrum*.

Table II: Zone of Inhibition of Aqueous extract of garlic on the dermatophytic fungi

Organism	Concentration (mg/ml)					
	Control (Griseofulvin400m)	25	50	100	200	400
<i>T.mentagrophyte</i>	5.0±7.1b	0.0±0.0	6.5±2.1b	11.0±1.0b	21.5±9.2	33.0±4.2
<i>T.rubrum</i>	8.0±0.0a	3.0±4.2	7.5±0.7a	10.0±0.0	24.5±0.b	29.5±6.4b
<i>T.tonsurans</i>	15.0±4.2	0.0±0.0	6.0±0.0	10.0±0.0	24.0±0.b	27.5±2.1b
<i>T.violaseum</i>	15.0±7.0b	0.0±0.0	6.0±0.0c	10.0±0.c	18.0±0.a	31.0±8.5

Each value is a Mean of standard error of three replicate. Means trailed by the same superscript across columns are not significantly diverse from each other.

In the current study, 133 samples were collected and were affirmative in direct microscopic examination (KOH). 60 were culture positive and 73 were culture negative. This gained the support of other studies; Bindu V. *et al.* (2002) , perceived in their work that in direct microscopy, positive cases was 64% and culture positive was 45.3%. (Singh and Beena, 2003) also stated 60.38% positive cases by microscopy and 44.6% cases were culture affirmative, while 53.38% did not present evidence of fungal presence through both direct microscopy and culture.

CONCLUSION

This study investigated the phytochemical properties and counter-dermatophytic activity of garlic aqueous extract. The isolation, identification of dermatophytes, collection and preparation of plant and extraction using aqueous solvent, phytochemical screening and antifungal activity determination, were carried out in this study. This research confirms that *Allium sativum* (garlic) aqueous extract encompass vital and vigorous phytochemical composites that successfully countered dermatophytic activities and thus can be exploited as

an alternate choice for the treatment of dermatophytes. *Allium sativum* (garlic) aqueous extracts had a noticeable proof in the inhibition of the organisms tested. Therefore, *Allium sativum* (garlic) has proven potency for anti-dermatophytic operations. A study more advanced than this is encouraged towards organic-based drug design using *Allium sativum* (garlic) to counteract dermatophytosis infections. Advanced assessment of the counter-dermatophytic possessions of garlic extracts opposition to other broad fungi is needed.

CONFLICT OF INTEREST: There was no conflict of interest at all.

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