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Potentials of Eucalyptus Tereticornis (Leaf and Stem Bark) For Antifungal Activities.

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ABSTRACT:
The potential of Eucalyptus tereticornis (leaf and stem bark) extracts was investigated for antifungal activities of some selected pathogenic fungi of vegetables. The test was carried out in vitro. Samples of pathogenic fungi of isolated from vegetables obtained from Market and farm in Sokoto metropolis and were tested for growth inhibition. The result indicated that the plant extracts were found to be effective in reducing the growth of the pathogens. The degree of inhibition was significantly higher ($p<0.05$) in E. tereticornis extracts. Acetone and MSW extracts were more effective in reducing the growth of the test fungi than aqueous extracts. A. niger was more sensitive to the extracts of the plant. These findings should be integrated in disease management strategies in order to minimise the use of fungicides which are costly and have deleterious effect on non target organisms.

KEYWORDS: Eucalyptus tereticornis,Plant, extract, pathogens, fungi

I. INTRODUCTION

Eucalyptus tereticornis is a species of tree native to eastern Australia. E. tereticornis has several common names. These include Forest Red Gum, Bastard Box, Blue Gum, Flooded Gum, Grey Gum, Mountain Gum, Queensland Blue Gum, Red Gum, Red Ironbark, Red Iron gum and Slaty Gum. The tree grows to a height of 20 to 50 metres with a girth of up to 2 metres. The species has a wide distribution, occurring over the widest range of latitudes of any Eucalyptus species. E. tereticornis has strong, hard and durable heartwood, with a density of about 1100 kg m−3. It is used for construction such as for railway sleepers [9]. The leaves of E. tereticornis are used in the production of cineole based eucalyptus oil. Essential oil extracted from Eucalyptus leaves contains compounds that are powerful natural disinfectants which could be toxic when used in large quantities [10]. Eucalyptus oil is readily steamed, distilled from the leaves and can be used for cleaning, deodorising, and in very small quantities in food supplements, especially sweets, cough drops, toothpaste and decongestants. It has insect repellent properties [17], and is an active ingredient in some commercial mosquito repellents [13]. All parts of Eucalyptus may be used to make dyes that are substantive on protein fibres (such as silk and wool), simply by processing the plant part with water. Eucalypts are lauded for their beneficial economic impact on poor population [22].

Incessant and extensive use of synthetic pesticides is posing serious problem to the life supporting systems due to their residual toxicity on humans [11]. It is estimated that hardly 0.1% of the agro-chemicals used in crop protection reaches the target pest, leaving the remaining 99.9% to the environment to cause hazards to non target organisms including humans [11]. In addition to causing harm to wildlife, pesticides that travel from their original location to another are known to cause harm to humans. Human exposure to pesticides has caused poisonings, the development of cancer and the deaths of between 20,000 and 40,000 people worldwide each year [19]. The recurrent and indiscriminate use of fungicides have posed serious threat to human health and to the existing human eco geographical conditions as some of those chemicals have already been proven to be either mutagenic, carcinogenic or teratogenic [5]. Many pathogenic microorganisms have acquired resistance to synthetic pesticides [30]. This seriously hinders the management of diseases of crops and agriculture products.

Eucalyptus leaves have been established for their potentials as powerful natural disinfectants that could be toxic when used in large quantities [10] and also possessed an active ingredient which is used in some commercial mosquito repellents [7] However its potentials as fungicides has not been document in Sokoto by majority of researchers, Therefore this research intends to explore the potentialities of the leaves and back of the plant for

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antifungal activity and to document and made the information available for the populace especially farmers and growers of vegetable for utilization for disease management by determining the antifungal efficacy of E. tereticornis extracts on growth of fungal pathogens of vegetables and also determine minimum inhibitory concentration (MIC) of the extracts on fungal pathogens. The research will also identify the best solvent for extraction of the plant samples.

II. MATERIALS AND METHODS

Preparation of plant leaf and bark extracts

The samples were washed thoroughly with running tap water and then rinsed with distilled water. The fresh plant materials were separately air-dried under the shade. The dried samples were pulverised to obtain a powder with the help of pestle and mortar. The powder samples were stored in airtight bottles.

Aqueous extraction

Aqueous extract was prepared using the method of [7] with slight modification, by allowing the mixture to stand for 24 hours instead of 48 hours due to higher room temperature (37^{o}C) in the study area. One hundred gram (100 g) of each powder sample was added to 1litre of distilled water in a sterile flask and mixed thoroughly. The mixture was left to stand for 24 hours then filtered through double layered muslin cloth. The filtrate was transferred into a 1000 ml beaker and evaporated on a hot plate at 40^{0}C. The residue obtained was then preserved aseptically in sterile bottles at 5^{o}C for further use.

Acetone extraction

The method of Alkhail [1] was used with slight modification by allowing the mixture to stand for 24 hours instead of 48 hours due to higher room temperature (37^{o}C) in the study area for the extraction. One hundred gram (100 g) of each powder sample was added separately to 1litre of acetone in a sterile flask and mixed thoroughly. The mixture was left to stand for 24 hours and then filtered through double layered muslin cloth. The filtrate was transferred into 1000 ml beaker and evaporated on a hot plate at 40^{0}C. The residue obtained was then preserved aseptically in sterile bottles at 5^{o}C for further use.

Millet Steeped Water (MSW) extraction

Fermented Milled Millet Steeped Water production was carried out by overnight steeping of 2 kg of pearl millet (Pennisetum glaucum) obtained from Sokoto Central Market. The steep water was then discarded and wet milling of the millet grains was carried out. Water was added to the milled materials to make thick slurry. The slurry was sieved to remove chaff. The slurry was allowed to ferment and sediment/decant for 3 hours [20]. The liquid top-layer was separated from the sediment at the bottom-layer. The liquid water collected in a 10 litre gallon as millet steeped water (MSW) and allowed to stand for 3 days before it is put into use. One hundred gram (100g) of each powder sample was added separately to 1litre MSW in a sterile flask and mixed thoroughly. The mixture was left to stand for 24 hours and filtered through double layered muslin cloth. The filtrate was transferred into 1000 ml beaker and evaporated on hot plate at 40^{0}C. The residue obtained was then preserved aseptically in sterile bottles at 5^{o}C for further use.

Mycelial growth inhibition

The antifungal efficacy of the plant extracts was tested in vitro against three test fungi isolated from vegetables. The poisoned food technique as described by Nene and [23]; and Sangoyomi [26]was used. The plant extracts were constituted to varying concentration with distilled water. The concentrations were constituted by dissolving 0.05 mg, 0.15 mg, 0.30 mg, 0.45 mg and 0.60 mg of each plant extracts in five millilitre (5ml) distilled in test tubes. The test tubes were thoroughly shaken to obtain homogeneous mixtures. The mixtures formed concentrations as 1%, 3%, 6% 9%, and 12% respectively. Each of the concentrations was dispensed per petri dish and 9ml of the media (molten PDA) was added to each of the extracts containing petri dishes resulting in PDA-extract mixtures. These were gently rotated to ensure homogeneous dispersion of the extracts and then allowed to solidify. Five-day old fungal culture is punched aseptically with a sterile cork borer of 7mm diameter. The PDA-extract mixture were inoculated at the centre of the Petri dishes with a 4mm diameter mycelia dish obtained from the colony edge of 7-day old pure cultures of each of the test fungi. Negative controls were set up using blank agar plates (no extracts) and inoculated with each test fungus, and the positive control consisted of the fungicide which was prepared according to the manufacturer's direction by dissolving 0.5g in 100ml of sterile distilled water. Three replicate plates of PDA-extracts per isolates were arranged in the incubation room at an ambient temperature 35±2^{0}C and radial growth was measured daily for 7days.

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Measurement of mycelial growth (diameter) (mm)

Colony diameter was taken as the mean along two directions on two perpendicular lines drawn on the reverse side of the plates. The effectiveness of the extract was recorded in terms of percentage inhibition [23]

$$\% \text{ Mycellial inhibition} = \frac{\text{Mycellial growth}_{(control)} - \text{Mycellial growth}_{(treatment)}}{\text{Mycellial growth (control)}} \times 100$$

Experimental design and Statistical Analysis

The experimental design was a complete randomised design (CRD) with three (3) replications. The data obtained from the study were statistically analysed using the SPSS statistical version 16. The data were subjected to analysis of variance (ANOVA) and means were compared by Duncan's Multiple Range Test (DMRT) at $p<0.05$ significant level

III. RESULTS

Antifungal Efficacy of E. tereticornis (Leaf) extract on growth of fungal Pathogens.

The inhibitory effect of acetone, aqueous and MSW on the growth of the fungal pathogens (Table 1) indicated that acetone extract reduced the growth of R. oryzae (52.22±0.38%) and A. niger (70.08±1.13%) which were significantly different $p<0.05$. The acetone extract had no inhibitory effect on growth of M. racemosus Aqueous extract had low inhibitory effect on growth of R. oryzae (7.71±0.38%) while there was no inhibitory effect on mycelia growth of A. niger and M. racemosus. MSW had high inhibitory effect on mycelia growth of A. niger (81.43±1.13%) but had no inhibitory effects on mycelia growth of R. oryzae and M. racemosus. The plant extracts at different concentrations indicated that 9% concentration had higher inhibition effect on mycelia growth of R. oryzae (39.26±0.50%) which was significantly different from the value obtained from 12%, 6%, 3% and 1%.

The plant extract had no inhibitory effect on mycelia growth of M. racemosus at all the different concentrations. The different concentrations had inhibitory effects on mycelia growth of A. niger 6% and 1% concentrations had the highest inhibitory effect 55.11±1.45% and 51.18±1.45% respectively. These were not significantly different at $p<0.05$. However, they were found to be significantly higher than the values obtained from the other concentrations. 12%, 3% and 9% concentrations had inhibitory values 50.12±1.45%, 48.37±1.45% and 47.73±1.45% which were not significantly different ($p<0.05$). There was an interaction between the fungi (R. oryzae and A. niger), extracts and the concentrations, but there were no interactions with M. racemosus.

Table 1: Antifungal Efficacy of E. tereticornis (Leaf) extract on growth of fungal pathogens.

Factor	Zone of Inhibition (%)		
	R. oryzae	M. racemosus	A. niger
Extract			
Acetone	52.22^{a}	---	70.08^{b}
Aqueous	7.71^{b}	---	0.00^{c}
MSW	0.00^{c}	---	81.43^{a}
SEM±	0.38	---	1.13
Concentratio(%)			
1	0.93^{e}	---	51.18^{a}
3	16.79^{d}	---	48.37^{b}
6	17.91^{c}	---	55.11^{c}
9	39.26^{a}	---	47.73^{b}
12	25.00^{b}	---	50.12^{a}
SEM	0.50	---	1.45

Means bearing different superscript along the same column within subclass differ ($p<0.05$).
MSW- Millet Steeped Water.
SEM- Standard Error Mean
--- no growth inhibition

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Antifungal Efficacy of E. tereticornis (bark) extract on growth of fungal pathogens

The result on Table 2 indicated that Acetone and MSW extracts of E. tereticornis (bark) reduced the growth of A. niger 78.39±0.85 and 82.71±0.85 respectively, while aqueous extract had no inhibitory effect on mycelia extension of M. racemosus and A. niger. Acetone and MSW had no significant effect on growth inhibition of R. oryzae and M. racemosus at $p<0.05$. The extracts at different concentrations indicated that there was slight reduction of growth of R. oryzae. There was growth inhibition at 9% concentration and the inhibitory value is 9.38±0.30, that was significantly different from the values obtained at 12% (4.19±0.30%), 3% (2.59±0.30%), 6% (0.09±0.30%). The extract had no effect at 1% concentration. 12% concentration had low growth inhibition on M. racemosus (11.00±0.52) while there was no inhibitory effect on growth of same organism at 1%, 3%, 6% and 9% concentrations respectively. High growth retardation of A. niger was observed in different concentrations with 12% having the highest value (55.68±1.10%) which was significantly different from what was obtained from the other concentrations. 9% (53.03±1.10%), 6% (52.60±1.10%), 3% (54.48±1.10%) and 1% (52.72±1.10%) respectively. There was interaction between organisms, extracts and the concentrations ($p<0.05$).

Table 7: Antifungal Efficacy of E. tereticornis (bark) extract on growth of fungal pathogens

Factor	**Zone of Inhibition (%)**		
	R. oryzae	**M. racemosus**	**A. niger**
Extract			
Acetone	7.23[a]	---	78.39[b]
Aqueous	2.51[b]	---	0.00[c]
MSW	0.00[c]	6.60[a]	82.71[a]
SEM	0.23	0.41	0.85
Concentration(%)			
1	0.00[d]	---	52.72[a]
3	2.59[c]	---	54.48[b]
6	0.09[d]	---	52.60[a]
9	9.38[a]	---	53.03[a]
12	4.19[b]	11.00[a]	55.68[c]
SEM	0.30	0.52	1.10

Means bearing different superscript along the same column within subclass differ ($p<0.05$).
MSW- Millet Steeped Water.
SEM- Standard Error Mean
--- no growth inhibition.

IV. DISCUSSION

The leaf and the stem bark extracts of E. tereticornis had no inhibitory effect on the mycelia growth of Mucor racemosus at different concentrations. The bark extract which had a slight inhibition at 12% concentration could be attributed to the difference in physiology and anatomy of the organism as facultative saprophytes also reported by Iwu et al. (1992). Hence the extracts might have served as a carbon source for the growth of the organism. Rhizopus sp displayed slight to moderate sensitivity with the leaf and the bark extracts of E. tereticornis at different concentrations, while high antifungal activity at different concentrations of same extracts was observed on growth of A. niger.

The mycelia growth of the test fungi differs in their levels of inhibition by the plant extracts of E. Tereticornis. This indicated that the plant extracts have the potentiality to reduce the mycelia growth of the test fungi, which also suggests that the plant possessed anti fungal properties. That could be due to the high concentration of tannins, saponins, flavonoid and alkaloid present in both the leaf and bark extracts of the plant. Tannin had been reported to prevent the development of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them [26]. Saponin has been reported to be active antifungal agents [15] . On the other hand, alkaloids are commonly found to have antimicrobial property and flavonoids are shown to inhibit growth of microbes which are resistant to antibiotics ([16].

The extent of fungal growth inhibition caused by all the plant solvent extracts varied. The highest inhibition of mycelial growth of the organisms was recorded from MSW extract followed by Acetone extract and the least was aqueous which could be because the ability of the solvents to extract phytochemical compounds differs. The disparity in

the level of efficacy of the solvent extracts was in line with reports [27]; [28]; [21] that worked on antifungal efficacy of some tropical botanical extracts in controlling various phytopathogens and obtained similar result. The inhibitory effect of MSW could be due to the presence of large number of viable lactic acid bacteria which help in inhibiting the growth of fungi. MSW is a fermented product which contains flavour and aromatic compounds, biomass proteins/amino acids, carbohydrates, vitamins and other products of respiratory/biosynthetic process like lactic acid, ethanol, acetylaldehydes, pyruvic acids, which help in altering the pH of the food to levels that do not favour the growth of pathogenic fungi [2]; [14]; [28]; [15]; [8]; [3]; [18]. Hence it proved to be effective in retarding fungal growth in this research.

V. CONCLUSION

The mycelial growth of all the three fungal pathogens differs in their levels of inhibition by the plant extracts of E. tereticornis and it extract was found to have high inhibitory ability. MSW was the most effective extract for antifungal activity, closely followed by acetone and the least was aqueous extract. A. niger was found to be the most sensitive pathogen to both the plant extracts.E. tereticornis should be utilised for its potentials for antifungal activities of vegetable pathogens.MSW should be utilised as the solvent for extraction because of its effectiveness as extracting solvent and its availability and affordability. The research should serve as baseline for further studies on industrial production of bio-fungicides in the management of fungal vegetable disease.

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