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Research Article

EXTRACTION OF BIOACTIVE SOLUTES IN *JATROPHA TANJORENSIS* (HOSPITAL-TOO FAR) LEAF: EFFECTS OF CO-SOLVENTS AND EXTRACTION TIME

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ABSTRACT

The e effect of co- solvents and extraction time on the extraction of bio active solutes in *Jatropha Tanjorensis* (hospital-too far) leaves was investigated in this work. Four solvent combinations, 50cm^{3}of water added to 50cm^3 each of ethanol, methanol, acetone and propylene glycol were used as extractants for the powdered leaves. The optimal soaking period for the extraction was examined by leaving powdered leaves in each solvent combination for 6, 24 and 72 hours with occasional shaking. While the bioactivity of the extracts was tested on bacteria isolates; Staphylococcus Spp., Proteus Spp., E- Coli Spp., and Pseudomonas Spp. The result revealed that the water- ethanol co solvent was the most effective after 72 hours soaking time as the growth of all the bacterial isolates studied were inhibited and gave better zones of inhibition. Although crude leaf extracts of *Jatropha Tanjorensis* inhibited the bacterial strains studied, solvent combination and soaking time affected the effectiveness of the extracts. as depicted by their zones of inhibitions, Thus, implying that the used co-solvents are good extractants of bioactive solutes (Phytochemicals) contained in the plant matrix, due to the formation of a solvent system of optimal polarity and conducive medium for the dissolution of the solutes.

INTRODUCTION

The plant *Jatropha Tanjorensis* belongs to the family-Euphorbiacea, which is a common weed of field crops, bush re-growth, roadsides and disturbed places in the higher rainfall forest zones of West Africa. It is commonly referred to as "Hospital –Too-Far", "Catholic Vegetable", "Iyana-ipaja", and "Lapalapa" by some locals (Iwalewa *et al*., 2005). The name 'Jatropha' was derived from the ancient Greek word "*latros*" (doctor) and "*tropha*" (feed) because of its medicinal applications as reported by Elbenri *et al*.(2013).

The plant consists of about 7,500 species which are drought-resistant perennial and multipurpose trees, similar to the cassava plant. It is a deciduous tree that sheds its leaves during the dry season, which is typical of all deciduous trees. When fully grown, its height is about 3-5m and remains productive for 30 to 50 years (Elbenri *et al*., 2008). It is used to stabilize soils against landslides and erosion prevention because of its tap root system. Other uses include fencing and consumption of the leaves as vegetable in most parts of Southern Nigeria. Recently, it has become a popular natural remedy against several ailments such as diabetes (Olayiwola *et al*., 2004), malaria infection and hypertension (Orhue *et al*., 2008). The increasing discovery of more medicinal plants have necessitated the need for increased scientific scrutiny of their bioactive solutes (Phytochemicals), in order to provide data that will help physicians and patients make wise decision before using them (Oyewole *et al*., 2007 and Ozolua *et al*., 2006). The medicinal properties exhibited by this plant is attributed to the presence of certain solutes (Phytochemicals) like alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins as reported by Ehimwenma and Osagie, (2007).

Therefore in recent times, there is greater emphasis on the recovery or extraction of high value-added products by using sustainable technologies. One of the ways of achieving this is the application of sub-and supercritical fluids (ScF). ScF can be applied as solvent for precipitation and micronization as reaction medium, mobile phase for chromatography (supercritical fluid chromatography-SFC) and as solvent for extraction. The most common solvents used are Carbon dioxide when polar components are extracted, but to enhance solubility,

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a co-solvent is added. The use of co-solvents such as ethanol-water to extract bioactive solutes have been reported by Parka *et al* (2007), who concluded that ethanol-water is a good co-solvent for the extraction of bioactive solutes in plant matrix. Several works have been done on the extraction of bioactive solutes in *Jatropha Tanjorensis* leaf in alcohols, acetone, water and propylene glycol respectively, but studies on the effect of co-solvency is yet reported. Hence, this work attempts to extract the bioactive solutes in *Jatropha Tanjorensis* leaf using co-solvents of water-alcohols, water-acetone and water-propylene glycol. The extracts will be used to test the resistivity of bacteria isolates such as. Staphylococcus Spp., Proteus Spp., E- Coli Spp., and Pseudomonas Spp. The optimal extraction period will also be determined.

METHODOLOGY

Study Area

This study was carried out in the School of Pharmacy Technicians, College of Health Science and Technology, Port Harcourt, suited at KM 4 Oro-Owo, Rumueme, Rivers State, Nigeria.

Collection and Preparation of Plant Material

The plant material (leaf) used in this study was harvested in the month of October, 2015 from the vicinity of a home garden in Alakahia Housing Estate, Port Harcourt, Nigeria after proper identification. The harvested leaves were air dried for some days, then further dried in an oven at 40^0C for 24 hours before grinding. The ground leaves were preserved in moisture-free, air tight laboratory containers before use. Ten grams of *Jatropha Tanjorensis* powder was transferred into three sets of four beakers each containing 50cm^{3}of water and 50cm^3 of ethanol, methanol, acetone and propylene glycol respectively. The solution was stirred vigorously for 10 minutes and left to stand for 6, 24 and 72 hours respectively with occasional shaking. The contents of the three sets of four beakers were then filtered, and the filtrate used to determine the antimicrobial potentials of the bioactive solute in *Jatropha Tanjorensis* leaf crude extracts.

Collection of Bacterial Isolates

The test organisms were sourced from Braith Waite Memorial Specialist Hospital (BMSH), Port Harcourt, Nigeria and brought to the Medical Laboratory Department of the College. The bacterial isolates include: Staphylococcus Spp., Proteus Spp., E- Coli Spp., and Pseudomonas Spp.

Preparation of Crude Antibiotic discs

A sterile whatman no 1 filter paper was punched using a perforator into 5mm diameter disc sizes. These discs were autoclaved for sterilization at 121^{o}c for 15 minutes after which the discs were allowed to dry. The crude leaf extracts (5-10ml) was transferred into the sterile Bijou bottle containing sterile disc, allowed to soak in the extract for 1 hour absorption, after which they were removed and allowed to dry.

Antimicrobial Assay of Extracts

Nutrient agar (3.4g) was added to 120ml distilled water in a flask. This was sterilized by autoclaving at 121^{o}c for 15 minutes. Each Petri disc was added a portion of the medium (20ml) and allowed to solidify. An isolate colony of each test organisms was sub-cultured on the nutrient broth and incubated at 30^{o}c for 30 minutes. This was then spread on the entire plate medium to ensure uniform growth. The sterilized crude leaf extract discs were placed against each plate containing the test organisms. The plates were then incubated immediately for 24 hours at 30^{o}c. Zones of inhibition were observed using a hand lens for proper magnification and measurement.

RESULTS AND DISCUSSIONS

Presentation of Results

The results obtained from the experiment was recorded and presented in tabular and graphical forms below:

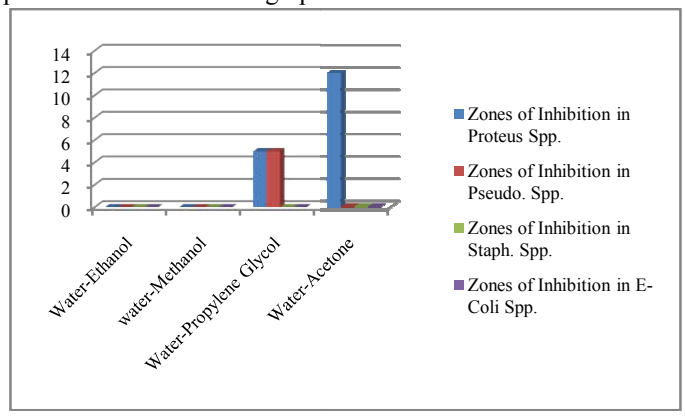

Figure 1 Antimicrobial Activities of Crude Leaf of *J. Tanjorensis* Soaked for 6 Hrs in Various Co-solvents.

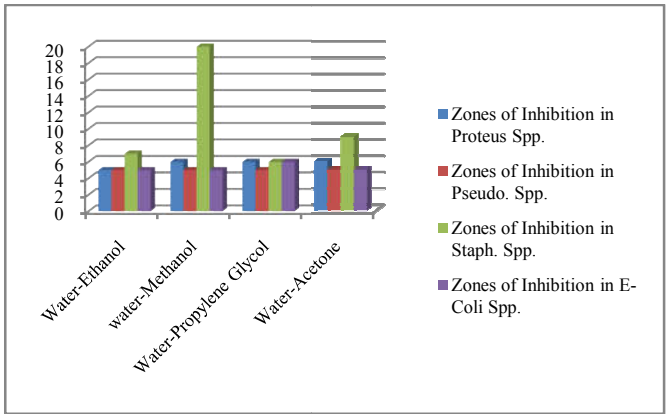

Figure 2 Antimicrobial Activities of Crude Leaf of *J. Tanjorensis* Soaked for 24 Hrs in Various Co-solvents.

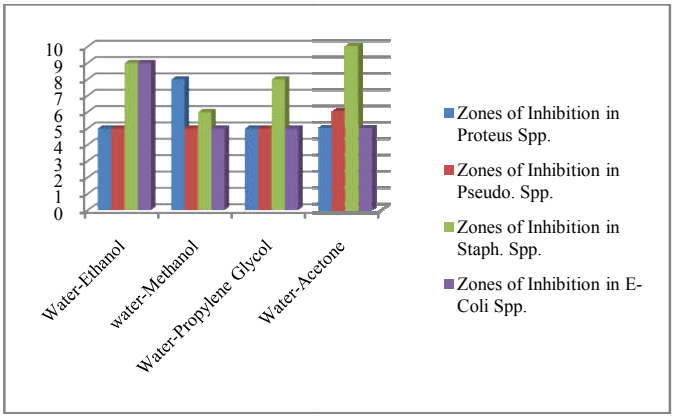

Figure 3 Antimicrobial Activities of *J. Tanjorensis* Leaf Soaked for 72Hrs in Various Co-Solvents.

Figure 1 reveals the antimicrobial activity of crude leaf extracts of *Jatropha Tanjorensis* obtained from co-solvents of equal volumes of water-ethanol, water-methanol, water-propylene glycol and water-acetone respectively. The results reveals that crude leaf extracts obtained from water-ethanol and water-methanol for 6 hours could not inhibit the growth of the isolated bacteria. A similar trend was observed in extracts obtained from water-propylene glycol and water-acetone soaked for 6 hours against *Staphylococcus Spp*., *E-Coli Spp*. and *Pseudomonas Spp*. except against Proteus Spp. The result of resistivity by the tested bacteria could be attributed to the soaking for a short period of 6 hours, which did not give enough time for the co-solvents to extract the bioactive solutes (Phytochemicals).

Again, the morphology and the location of the extractable solutes in the plant material is another contributing factor. According to Isecleman (2004), and Miha (2013), if the desired extractable solute is on the surface of the plant material, generally extraction rates will be high. However, when the desired extractable solutes are deeper in the plant material, it takes a longer or more time for extraction to take place. It could therefore be inferred that the extractable solutes (Phytochemicals) were not present on the surface of the plant material, but are rather deeper in the plant material. Hence, short time of 6 hours was not good enough for extracting the bioactive solutes in the plant material into the used co-solvents. Furthermore, the short period of 6 hours could not allow for mass transfer of the bioactive solutes into the solvents used, hence the great resistance by the isolated bacteria to the crude leaf extracts.

Figures 2 and 3 showcase the antimicrobial activity of the crude leaf extracts soaked for a longer period of 24 and 72 hours respectively. The result reveals that the crude leaf extracts inhibited the growth of all the bacterial isolates studied, and gave better zones of inhibitions. This result was in agreement with the works of Fred et al (2009), Sekaran (1998) and George *et al*.(2014). However, these zones of inhibitions varied between 5mm-20mm. The inhibitory effects could be attributed to the longer soaking period unlike the previous crude leaf extract. Soaking for a longer period allows the desired extractable bioactive solutes (phytochemicals) which are deeper in the plant material to be extracted or leached. Furthermore, the non complex structure of the plant material which does not have the desired extractable solutes deeper inside to pose greater resistance to extraction could be implicated. In such cases, mass transfers of extractable solutes are very possible.

The nature, particle size and porosity of the pulverized *Jatropha Tanjoresis* leaf are another contributing factor. According to Beckman (2004), mass transfer of bioactive solutes (Phytochemicals) depends on the aforementioned factors. The pulverized or powdered form and smaller particle size of the leaves, made it easy for the solvents to penetrate and extract the bioactive solutes (Phytochemicals). Again, the nature and type of co-solvents used also accounted for the mass transfer of the bioactive solutes into the solvents. The co-solvents of water-alcohols (water-ethanol and water-methanol) are weakly acidic. This slightly acidic condition enables good extraction and solubility of these bioactive solutes, since photochemicals can be activated in acidic medium. Furthermore, absence of steric hindrance by the molecules of the co-solvents (which does not possess highly branched groups to affect the position of equilibrium) favored strong intermolecular interaction between the antimicrobial solutes and the water-alcohol mixture (Jack, 2005).

Solubility of the solutes and the ability of the co-solvents to solubilize the solute are also implicated. Solubilization is a function of dielectric constant and polarity which plays a very big role in drug solubility both in organic and in-organic solvents, and affects pharmaceutical process of extraction. Mohammad et al (2010) observed that changes in dielectric constant of a medium have a dominant effect on the solubility of ionizable solutes in which higher dielectric constant can cause more ionization of the solutes and results to more solubilization. The dielectric constants of the individual solvents are as follows: water (80), methanol (33), propylene glycol (32.1), ethanol (25) and acetone (21) respectively. Water is most polar among these solvents, while others are semi-polar. On combining these solvents with water, an optimal dielectric constant is formed which could enable dissociation of different polar solutes (Phytochemicals) in the plant matrix and enhance the mass transfer of these bioactive solutes into the co-solvents. This implies that at lower dielectric constant, there is a high potential of the solvent to extract or leach more of the bioactive solutes into the extractants (Goli *et al*., 2012). Again, differences in polarities of the solutes affect the solubility of bioactive solutes. The water-alcohol, water-acetone and water-propylene glycol co-solvents gave an interesting finding that polar solvents are able to enhance the mass transfer of bioactive solutes since fractionazation between the less polar solute and the more polar solutes are possible (Markom, 2007). Montanes *et al* (2008), reported that co-solvents have the potential to extract bioactive solutes from plant materials by forming a solvent system of optimal polarity. This enables the solutes in *Jatropha Tanjorensis* leaf to be more soluble and thus, accounted for the observed results in 24 and 72 hours soaking periods.

Finally, the inhibition of bacterial growth by crude leaf extracts of *Jatropha Tanjorensis*, could be attributed to the production of physiological actions on the body of the isolated bacteria. These solutes inhibit nucleic acid, protein and membrane phospholipids biosynthesis (Almazini, 2007). This partitions the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structure to render them more permeable, and eventually lead to death (Shirsat, 2008 and Almazini, 2007).

CONCLUSION

The crude leaf extracts of *Jatropha Tanjorensis* obtained from co-solvents were effective against the bacterial strains studied, as depicted by their zones of inhibitions. The water-ethanol optimum was most effective extract at extraction period of 72 hours. Thus, implying that the used co-solvents are good extractants of bioactive solutes (Phytochemicals) contained in the plant matrix, due to the formation of a solvent system of optimal polarity and conducive medium for the dissolution of the solutes. The efficacy of the crude leaf extract could be enhanced by biotechnology. However, it is recommended that further work be done to isolate and purify the bioactive solutes in *Jatropha Tanjorensis* leaf extracts using various extraction

solvents with a view to characterizing as well as evaluating their safety or toxicity for human and other animal use.

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