Research Article

Chemical and Anti-tubercular Screening on the Leaves of *Jatropha multifida* Linn

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Abstract

The study was conducted to extract the leaves of *Jatropha multifida* Linn using ethanol, hexane, dichloromethane, ethyl acetate, n-butanol, and water; determine the presence of alkaloids, tannins, flavonoids, anthraquinones, saponins, cardiac glycosides and cyanogenic glycosides in the extracts using the Test Tube Method. Test the inhibitory activity of the extracts against *M. tuberculosis* H37Rv using Microplate Alamar Blue Assay. Phytochemical screening of the extracts revealed the presence of alkaloids, tannins, flavonoids, saponins, cardiac glycosides and cyanogenic glycosides. Results indicate that at 128µg/mL, sample extracts inhibited the growth of *M. tuberculosis* H37Rv. Comparing the activity of the extracts, hexane gave the highest anti-tubercular activity.

Keywords: Phytochemical, *Mycobacterium tuberculosis*, *Jatropha multifida* Linn

Introduction

The Philippines is known for its beauty because of the wide variety of plants and trees, which are available in unlimited resources and numbers, their size, vary, coming from the smallest family of plants to the towering species of trees. Unfortunately, not more than one half of these plants were known in terms of its chemical and bioactive constituents. Due to this reason, many plants and various types of trees were subjected in experimentation thru phytochemical screening and eventually thru chemical and bioactivity studies.

One of the major problems that human is being faced with is the fight against diseases and the pursuit to cure them. Prevention and control is hard to achieve because they are airborne and can be found almost everywhere. Tuberculosis (TB) is a worldwide pandemic. TB remains a major public health in the Philippines. According to World Health Organization's (WHO's) Global TB Report 2008, the Philippines ranks ninth on the list of twenty two high-burden TB countries in the world. Approximately 145 persons of every 100,000 population have active TB or whose sputum are positive for the TB bacilli. Worse, most of them belong to the economically productive age group of 15 to 54 years old. TB is the sixth leading cause of illness and also the sixth leading cause of deaths among Filipinos.

Nowadays, people are looking forward to cure their ailments and the pain they endure. Through time people resorted to drugs and medicine to heal their own

respective ailments. Despite of the developments in the Philippines, many citizens are still below poverty line and cannot afford to buy branded high priced medicines and high cost of medication. Alternative treatment is an immediate need of the society; the very valuable answer to this undertaking is the pharmacological and economic values of plants.

Further, traditional healing applications of herbal and medicinal plants have always been used on naturopathic clinicians of herbolarios in primitive medicine majority of which have no scientific bases. Thus, there is a need to conduct studies on chemical and bioactivities of certain plant species to prove the effectiveness of their traditional healing applications and health benefits.

Several species of the Euphorbiaceae family (spurge family) like *Jatropha* species, are used in traditional folklore medicine to cure various ailments [5, 6]. Research has been focused either on bioactivities of the plant extracts, or on isolating a special class of natural products [8,9,10,12]. The plant in study, *Jatropha multifida* Linn., is locally known as "*tubang amerikano*". In East Java, it was named as "*jodium*"; "*jarak gurita*" or "*jarak cina*" in Indonesia; "*manaku*" in Malaysia; "*kuthinerval*" in Tamil; and "*bhadradanti*" in Sanskrit. It is planted throughout the Philippines for ornamental purposes, and as hedge [11]. Known also as "*Mana*," this plant has been used for scabies, wounds and ulcers. The plant is used by the traditional healers as abortifacient, purgative, emetic, carminative, cathartic and tonic. Although the plant can be of great value, it is

yet to be evaluated with regard to physical, biological and chemical properties that have a bearing on its ultimate and successful formulation into a stable and effective pharmaceutical product [1,2,3,4]. This study will focus on the extraction, phytochemical and anti-tubercular screening of *J. multifida* L. to discover its properties for possible utilization as a drug.

Materials and Methods

The matured leaves of *Jatropha multifida* Linn. were collected. The leaves were washed thoroughly with water and air dried for 20 days. The air-dried leaves were ground. Herbarium specimen of the plant was prepared consisting of leaves, stem, flowers and fruits for proper identification. The specimen was brought to the University of Santo Tomas Herbarium. It was identified and given an accession number USTH 4772.

Two thousand three hundred grams ground air-dried leaves of *J. multifida* were subjected to cold percolation with three-liters of distilled technical grade ethanol at room temperature for 24 hours. The ethanol extract was drained and collected in amber bottles. Fresh ethanol was added in the percolator for another 24 hours and the process was repeated four times. Twenty liters of technical grade ethanol was expended in the extraction process.

The drained ethanol extract was concentrated in *vacuo* with the use of Buchi rotary evaporator set at 40-45°C until a syrupy consistency is attained. The crude ethanolic extract has a total weight of 264.00 grams. The crude ethanol extract was dissolved in distilled water and partitioned with distilled hexane. The hexane layer was treated with anhydrous sodium sulfate and filtered. The filtrate was concentrated in *vacuo* at 45°C until a syrupy consistency is attained. One hundred five grams (83.00g) of the concentrated hexane extract was obtained. The aqueous layer was extracted exhaustively with dichloromethane. The dichloromethane layer was treated with anhydrous sodium sulfate and filtered. The filtrate was concentrated in *vacuo* at 45°C until a syrupy consistency is attained to give 1.50 grams. The aqueous layer was further extracted with distilled ethyl acetate. The ethyl acetate layer was dried with anhydrous sodium sulfate and filtered. The filtrate was concentrated in *vacuo* at 45°C and attained 0.80 gram. Finally, extraction of the aqueous layer with n-butanol was done. The butanol layer was dried with anhydrous sodium sulfate and filtered. The filtrate was concentrated in *vacuo* at 45°C to yield 22.00 grams.

Test Tube Method was used in the phytochemical screening of the extracts. Test for Alkaloids. Five milliliters of 2M HCl is added to 2- milligram of the extract. The mixture was heated for five minutes with stirring and then cooled. About 0.5 g of NaCl was added. The residue was stirred, filtered and washed with enough 2M HCl to bring the filtrate volume to five milliliters.

One milliliter of the filtrate was treated with 2-3 drops of Wagner's reagent. A positive result was indicated by the formation of a brown precipitate. Likely, 1 mL of the filtrate was treated with 2-3 drops of Mayer's reagent. Formation of white precipitate indicated a positive result. Test for Tannins. Five drops of 10% NaCl solution was added to 2 mg of the plant extract. It was then filtered and the filtrate was divided into three equal parts. One portion was used as a control while an aqueous solution of tannic acid was used as reference standard. The second portion was treated with 3 drops of gelatin salt solution. Formation of a precipitate signified the presence of tannins. The third portion was treated with ferric chloride test reagent. Formation of a blue-black color signifies the presence of hydrolysable tannins, while a brownish-green color may indicate the presence of condensed tannins. Test for Flavonoids (Bate-Smith and Metcalf Test for Leucoanthocyanins). Ten milliliters of 80% ethanol was added to 5 mg of the plant extract and was filtered. The filtrate was divided into four equal parts with one portion served as control. One portion of the filtrate was treated with 0.5 mL concentrated HCl and observed for any color change. It was then warmed for 15 minutes on a water bath. Further change was observed for the next hour. A strong red or violet color revealed the presence of Leucoanthocyanins. Shinoda Test. The other portion was added with magnesium powder and a few drops of concentrated HCl. Orange, pink, red to purple color appeared when flavones, flavonols, the corresponding 2,3 dehydroderivatives and or xanthenes are present. Addition of *tert*-butyl alcohol before adding the acid was done to avoid violent reaction. The colored mixture was dissolved into the upper phase. Zinc was added instead of magnesium. Flavanones gave a deep red to magenta colors while flavones and flavonols showed pink to magenta colors or no color at all. The third portion of the filtrate was treated with concentrated H₂SO₄. When flavones and flavonols dissolved in concentrated H₂SO₄, a deep yellow solution is visible. Chalcones and aurones produce red to red-bluish solutions. Flavanones gave orange to red colors. Test for Saponins (Froth Test). One gram of the bark of *Entadea phaseolides* was extracted with 10 mL of ethanol. This was used as the standard extract. Two milliliters of the plant extract was transferred into a test tube and 2 mL of the *Entadea phaseolides* extract into another test tube. Ten milliliters of distilled water was added to each test tube, stoppered and shook vigorously for 30 seconds. They were allowed to stand for 30 minutes. A "Honey Comb" froth above the surface of the liquid that persisted for 30 minutes, a positive indication of the presence of saponins. The result was compared to *Entadea phaseolides* extract. Liebermann-Burchard Test. Five milligram of the extract defatted with 10 mL of hexane or petroleum ether. Ten milliliters of chloroform was added and was stirred for

five minutes. The mixture was allowed to stand and the chloroform extract was taken off. The chloroform part was dried by filtering it thru 100 mg of anhydrous sodium sulfate. The filtrate was divided into two portions. One portion was used as control while the other portion was treated with 3 drops of acetic anhydride and 1 drop of concentrated sulfuric acid. Any immediate change in color was an indication of a positive result. It was allowed to stand for an hour to observe further color changes. Test for Cardiac Glycosides (Keller-Kiliani Test). Two milligram of the plant extract was defatted by titrating with hexane. The hexane extract was decanted off and the treatment was repeated until most of the colored pigments were removed. The defatted residue was warmed over a water bath to remove the residual hexane. Three milliliters of FeCl_3 was then added and stirred. One milliliter of concentrated H_2SO_4 was added cautiously with the test tube in an inclined position. The mixture was allowed to stand and was observed for any coloration at the interface. A reddish-brown color, which may turn into blue or purple, indicated the presence of 2-deoxysugars. Kedde Test. Two milligrams was treated with 2 mL of CHCl_3 and mixed well. The chloroform layer was taken and treated with 4 drops of Kedde reagent. A blue violet color indicates the presence of cardiac glycosides. Test for Cyanogenic Glycosides (Guignard Test). Two milligram of the plant extract was placed in a test tube. It was moistened with enough water and then a few drops of CHCl_3 were added to enhance enzyme activity. The test tube was stoppered with a cork, from which was suspended a piece of picrate paper. The paper strip was not being allowed to touch the inner side of the test tube. The test tube was warmed at $35-40^\circ\text{C}$. A change from yellow to brick red indicated a positive result; the intensity or shade of the red color produced within 15 minutes measured the relative concentration of the cyanogenic glycosides. The absence of a color change after three hours signified a negative result. The same procedure was also performed on the leaves of the Tapioca plant (kamoteng kahoy) which served as the positive control.

The inhibitory activity of the sample extracts against *M. tuberculosis* H₃₇Rv was determined using the Micro-plate Alamar Blue Assay (MABA) courtesy of Prof. Scott G. Franzblau, Ph.D. of the Institute for Tuberculosis Research, Center for Drug Discovery, University of Illinois, Metropolitan Avenue, Chicago, IL USA [7]. Five milligrams of the extract was used for the assay. The percent inhibition was determined at concentrations 128 $\mu\text{g/mL}$, 64 $\mu\text{g/mL}$, 32 $\mu\text{g/mL}$, and 16 $\mu\text{g/mL}$ using rifampin as standard.

Results and Discussion

Phytochemical screening of the extracts revealed the presence of alkaloids, tannins, flavonoids, saponins, cardiac glycosides and cyanogenic glycosides. Table 1

gives the results in the phytochemical screening of the extracts.

The formation of white precipitate using Mayer's reagent and the formation of brown precipitate using Wagner's reagent indicate the presence of alkaloids. Tests were heavily turbid which suggest that tertiary alkaloids were present. The extracts contained tannins. This was shown by the formation of a jelly-precipitate using the Gelatin test and a brownish-green precipitate in Ferric Chloride test signifies that condensed tannins are present. Likely, flavonoids were present in the extracts due to the formation of strong red color using the Bate-Smith and Metcalf test for leucoanthocyanins. Using the Shinoda test, with the addition of zinc powder, flavonoids were detected due to the changing of the solution from deep red to magenta colors. Flavonoids were also detected using the sulfuric acid test when the color of the solution turned into orange red. Saponins were present due to the formation of froth having a height of 1 cm which lasted for more than 30 minutes using the Froth test. Using the Liebermann-Burchard test, immediate formation of dark green precipitate was apparent and the color changed to brown after one hour of observation which signified the presence of saponins. Furthermore, cardiac glycosides were present due to the formation of reddish-brown coloration of the solution using the Keller-Kiliani test. The observed result indicate the presence of 2-deoxysugars. The presences of cardiac glycosides were observed in the Kedde test due to the change in color of the solution into blue-violet.

The extracts were subjected to the Microplate Alamar Blue Assay to determine antitubercular activity. Different concentrations were prepared – 128 $\mu\text{g/mL}$, 64 $\mu\text{g/mL}$, 32 $\mu\text{g/mL}$, and 16 $\mu\text{g/mL}$. Results revealed that the inhibitory growth activities of the different extracts were enhanced at of 128 $\mu\text{g/mL}$ concentration (Table 2).

Table 1. Phytochemical Screening of the Extract

Phytochemical	Confirmatory Test	Result
Alkaloid	Mayer's test	+++
	Wagner's test	+++
Tannins	Gelatin test	+
	Ferric Chloride test	+
Flavonoids	Bate-Smith and Metcalf test	+
	Shinoda test	+
	Sulfuric Acid test	+
Saponins	Froth test	+
	Liebermann-Burchard test	+
Cardiac glycosides	Keller-Kiliani test	+
	Kedde test	+
Cyanogenic glycosides	Guignard test	+

Legend:

+ slight turbidity; ++ definite turbidity; +++ heavy turbidity; – no precipitate

Fig.1. Percent Inhibition of the extracts at 16µg/mL against *M.tuberculosis*. The percent inhibitory activity of the extracts at 16µg/mL were shown in figure 1. The crude ethanolic extract showed 32 % activity. The butanol extract displayed 25% activity while 22% inhibitory activity was exhibited by dichloromethane extract. Ethyl acetate and hexane gave 13% and 23 % activity respectively.

Fig.2. Percent Inhibition of the extracts at 64µg/mL against *M.tuberculosis*. The percent inhibitory activity of the extracts at 64µg/mL was shown in figure 2. The crude ethanolic extract showed 47 % activity. The butanol extract displayed 9% activity while 23% inhibitory activity were exhibited by dichloromethane extract. Ethyl acetate and hexane gave 14% and 30 % activity respectively.

Fig.3. Percent Inhibition of the extracts at 64µg/mL against *M.tuberculosis*. The percent inhibitory activity of the extracts at 64µg/mL was shown in figure 3. The crude ethanolic extract showed 73% activity. The butanol extract displayed 8% activity while 41% inhibitory activity was exhibited by dichloromethane extract. Ethyl acetate and hexane gave 29% and 62% activity respectively.

Fig.4. Percent Inhibition of the extracts at 128µg/mL against *M.tuberculosis*. The percent inhibitory activity of the extracts at 128µg/mL was shown in figure 4. The crude ethanolic extract showed 80% activity. The butanol extract displayed 17% activity while 60% inhibitory activity was exhibited by dichloromethane extract. Ethyl acetate and hexane gave 45% and 92% activity respectively.

Table 2. Percent inhibition of the Extracts against *M. tuberculosis* w

Extract	Percent Inhibition			
	128 µg/mL	64 µg/mL	32 µg/mL	16 µg/mL
Ethanol	80±0.50	73±0.78	47±0.67	32±0.89
Butanol	17±0.55	80±0.67	9±0.58	25±0.45
Dichloromethane	60±0.68	41±0.58	23±0.69	22±0.65
Ethyl Acetate	45±0.66	29±0.68	14±0.76	13±0.87
Hexane	92±0.90	62±0.95	30±1.50	23±0.45
Refampin	100±0.25	100±0.15	100±0.25	100±0.10

Conclusion

Phytochemical screening of the plant showed the presence of alkaloids, tannins, flavonoids, saponins, cardiac glycosides and cyanogenic glycosides. The plant extracts inhibited the growth of *M. tuberculosis* at different concentrations. The inhibition activity was exhibited much at 128µg/mL concentration.

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