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Research Article

Pharmacognosy for Drug Discovery

Pharmacognostic Study of Some Phytochemically Unexploited Floristic Trees of Hathwa, Gopalganj (Bihar)

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Abstract: Pharmacognosy of Indian plants has been a perk for studies and medicinal researches. Hathwa has been blessed with Flora with extraordinary nutritional and medicinal benefits. Plants of *Moringa oleifera* (Lam.), *Holarrhena antidysenterica* (Roth)Wall, *Erythrina indica* (Lam.) were found to be present at outskirts of Hathwa region of Gopalganj Bihar, They have been traditionally used in several disorders faced by human body but the stringent record and detailing is missing and this research was accomplished to relate their antimicrobial activities with their phytochemical entities. The plant extracts of bark was chosen because of its abundance presence and easy availability. The extracts were found positive for phenol, quinine, steroids, protein and amino acids The time-kill kinetics profile of extract of bark of *H. antidysentrica* (Roth) against the test organisms *K. pneumoniae, P. aeruginosa* and *V. cholerae* at the test concentrations of *Moringa oleifera* showed reduction in number of viable cells over the first 4, 8, and 12 hours *P. aeruginosa,* and *V. cholerae,* respectively, followed by a gradual rise up to the 10thh and 14thh for *K. pneumoniae* when compared to the control (organisms without antimicrobial agent).The time-kill kinetics profile of extract against the test organisms; at test concentrations studied showed reduction in number of viable cells over the first 2, 6, and 24 hours *K. pneumoniae, P. aeruginosa*, and *V. cholerae* respectively, followed by a gradual rise up to the 24thh for *P. aeruginosa* and *V. cholerae*and 12thh for *K. pneumonia* when compared to the control. Therefore from our research it was concluded that *Moringa oleifera* (Lam.), *Holarrhena antidysenterica* (Roth)Wall, *Erythrina indica* (Lam.) plants present at the outskirts of Hathwa region possess antimicrobial properties with important nutritional values. The aim of our research was to target some crucially important plants of Hathwa Gopalganj Bihar to study their antimicrobial potential, and this research targeted to explore the Phytoconstituents of those used plants which made them potent for antimicrobial activity.

Keywords: Phytochemical constituents, Time Kill Assay, Physicochemical analysis, Pharmacological activity.

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1. INTRODUCTION

The products derived from several herbs and plants, being a source of multifunctional curing agents and bioactive compounds, are relatively considered safe for consumption. According to the Food and Agriculture Organization's (FAO) report, about 70-80 % of the world's population, especially in developing countries, relies on herbal medicine to prevent and cure diseases [1]and about 25% of the synthesized drugs are manufactured from medicinal plants [2]**.** Increased demand for food to tackle hunger and malnutrition problems has been pertinent in developing countries over the last few decades. In Asian and African countries, the vast majority of the population suffers from malnutrition because of the deficiency of essential nutrients in foods. *Moringa oleifera* Lam. (syn. *M. pterygosperma*) belongs to the family *Moringaceae*, commonly known as the 'drumstick' or 'horseradish' tree. It is an affordable and readily available source of major essential nutrients and nutraceuticals, and it has the potential to eradicate malnutrition[3] **.**The *Moringa* is often considered as important famine food because of its high resistance to drought and arid conditions owing to their tuberous roots.[4]Almost each and every part of *Moringa* tree is useful for medicinal, functional food preparations, nutraceuticals, water purification, and biodiesel production; including roots, leaves, flowers, green pods, and seeds [5]**.** The other chosen Tree in this research is *Holarrhena pubescens*Wall. ex G. Don(Apocynaceae), commonly known as Kutaja", is an important plant used in indigenous systems of medicine as remedy for bronchitis, hematuria, spermatorrhoea, epilepsy, asthma, piles, leprosy, eczema, diarrhea, fevers and jaundice[6,7] Various parts of *H. antidysentrica* (Roth.) Wall have been reported to possess antibacterial activity**.** The bark has been reported to possess astringent and antidiarrheal properties[10]. Leaves of the plant are used to cure scabies[11.] *H. antidysenterica* (Roth.)Wall. is currently being used in the treatment of various disease conditions without standardization. However, a major constraint which has hindered the acceptance of alternative medicine is the lack of documentation and stringent quality control with this backdrop; it is of prime importance to make an effort towards standardization of the plant material. The process of standardization is achieved by stepwise pharmacognostic studies[12]which in turn help to identify and authenticate plant material. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established. *Erythrina indica* (Lam.) is also one amongst the unstudied plants found in the regions of forest plains as well as some outskirts of Hathwa; Gopalganj. *Erythrina indica* (Lam.) belongs to the family of Fabaceae (Legume family). It possess many useful entitlements including one said to destroy pathogenic parasites and relieve joint pain; the juice from the leaves is mixed with honey and ingested to treat tapeworm, roundworm and threadworm in India; women used to take this juice to stimulate lactation and menstruation; it is commonly mixed with castor oil to treat dysentery; a warm poultice of the leaves is applied externally to relieve rheumatic joints; and the bark is used as a laxative, diuretic, and expectorant. In a very few researches reported as antibacterial [13] [14]active against Cytotoxicity [15]**.** Analgesic and anti-inflammatory [16]**.** . The aim of our research was to target some of those crucially important plants of Hathwa Gopalganj Bihar to study their antimicrobial potential, and this research targeted to explore the Phytoconstituents of those used plants which made them potent for antimicrobial activity.

2. MATERIALS AND METHODS

2.1 Physiochemical and proximate analysis of tree bark

Moisture content was determined gravimetrically by drying the samples in an oven at 100°C to a constant weight. The dried barks from the above tree sample were subjected to other chemical analyses. Crude protein was determined in accordance with the Kjeldahl method (Method No. 978.04)**.** Crude fat was determined in accordance with the Soxhlet extract method using petroleum ether as the extract agent (60-80°C) (Method No. 930.09)[18]. Ash content was assayed by incinerating the samples in a muffle furnace at 550°C (Method No. 930.05)[19.]

2.2 Extraction of Phytochemical Constituents from tree bark

About 500g air dried powder of the plant materials of *Erythrina indica*(Lam.)**,** *Moringaoleifera*Lam.*,* and *Holarrhena antidysenterica* (Roth.)Wall. was extracted successively with the following solvent in Soxhlet extractor, and identified as fractions 1-3 as shown below; n-hexane-Fraction-1, Ethyl acetate-Fraction-2, and Methanol fraction-3. The extracts were made in the concentrations of 70% (150 ml) of methanol used for 25 gm of plant powder, 100 percent 150 ml of n –hexane, 100% Ethyl acetate (150ml) for other plant powders. Every time before extracting with the next solvent, the plant material was dried in a hot air oven below 500°C. N-hexane, methanolic, and ethyl acetate extracts were concentrated by distilling off the solvent and then evaporating to dryness. The yield after evaporation was 5 gm after all the phenomena was worked out the extract obtained contained minimal amount of moisture,the extracts were subjected to qualitative tests for the identification of various phyto-constituents.

2.3 Phytochemical Screening of the extract

The extracts obtained from the above process were then carried out for the phytochemical analysis. The phytochemical analysis was carried out for different bioactive compound determination using different standard procedures. This analysis was carried out in the similar way for Ethyl acetate, n – hexane, methanol extracts. The Alkaloid test was done by Mayer's test. For the test 1 ml of plant extract was mixed with 3ml of ammonia solution and the mixture was allowed to stand for a few minutes. Then 10 ml of chloroform was added to the same tube. The tube was shaken and the content was filtered. The chloroform was evaporated by keeping the tubes in a water bath. Then 3ml of Mayer's reagent was added to the tube. The result interpretation is by the formation of cream colour precipitate indicating presence of alkaloids. [20]For the test of Amino acids by method mentioned by Humphrey *et al.*[21]1ml of plant extract was treated with 0.5ml of the Ninhydrin reagent (0.25%). The content was boiled for a few minutes. The result interpretation is by the formation of blue colour indicating presence of amino acid in the extracts. The flavonoid was determined as an Alkaline Reagent test. For this, 1ml of plant extract was treated with a dilute sodium hydroxide solution that leads to the appearance of intense

yellow colour which fades away after addition of a few drops of dilute acid. The result is interpreted as positive when the colour disappears indicating presence of flavonoids. This test was followed by a test of Glycosides Keller-Killiani method was used for determining the test result. In the test 1 ml of plant extract was treated with a few drops of glacial acetic acid and ferric chloride solution and this content was mixed thoroughly. Next concentrated sulphuric acid was added to the tube and the tube was observed for the formation of two layers that is lower reddish brown layer and upper acetic acid layer. The result interpretation is done after the upper layer turns bluish green indicating the presence of glycosides in the sample [22] The presence of phenols in the extract was assessed through Ferric chloride test. 1ml of plant extract was treated with 3-4 drops of ferric chloride solution. The positive result is indicated by the formation of a bluish black colour indicating the presence of phenols. Test for the presence of protein was carried out by Xanthoprotic test. In this test 1 ml plant extract was treated with few drops of concentrated nitric acid. Formation of yellow colour is interpreted as the positive result for the test. Further Steroid test was carried out Liebermann Burchard test. For this 1ml of plant extract was mixed with 10 ml of chloroform. Then equal volume of concentrated sulphuric acid was added to the tubes. This leads to formation of two layers, the upper layer turns red and the sulphuric acid layer showsgreen fluorescence and this indicates the presence of steroids. Quinine was estimated by Virk *et al.*[23].

2.4 Time-Kill Kinetics Assay

Time-kill kinetics of methanol extracts of *Erythrina indica* (Lam,), *Moringa oleifera* Lam., *and Holarrhena antidysenterica* (Roth.) Wall. *volvacea* was carried out following the procedure described by Virk et al. [10] From the methanolic extract three different concentrations 500µl 1000µl 1500µl was taken and an inoculum size of CFU/ml was added and incubated at 37°C. Aliquots of 1.0 ml of the medium were taken at time intervals of 0, 1, 2, 3, 4, 5, 6, 12, and 24 h, for bacteria, and inoculated aseptically into 20 ml nutrient agar and incubated at 37°C for 24 h. A control test was performed for the organisms without the extracts or reference antibiotic. The colony forming unit (CFU) of the organisms was determined. The procedure was performed in triplicate (three independent experiments) and a graph of the log CFU/ml was plotted against time.

3. RESULTS AND DISCUSSION

3.1 Preliminary phytochemical analysis of crude plant extracts of Erythrina indica (Lam.), Moringa oleifera (Lam.) and Holarrhena antidysenterica (Roth.)Wall.

Table 1. Phytochemical study of plant extracts *H. antidysentrica*, *Erythrina indica* and *Moringa oleifera*

Plant Constituents	*H. antidysentrica* (Roth.)Wall			*Erythrina indica* (Lam.)			*Moringa oleifera* (Lam.)		
	Ethanolic Extract	Petroleum Ether Extract	Aqueous Extract	Ethanolic Extract	Petroleum Ether Extract	Aqueous Extract	Ethanolic Extract	Petroleum Ether Extract	Aqueous Extract
Steroid	+	-	+	+	-	+	-	+	+
Phenol	-	-	+	-	+	-	+	-	+
Amino acid	+	-	-	+	+	+	-	-	-
Protein	-	+	+	+	-	+	-	+	-
Glycoside	+	-	+	-	+	-	+	-	+
Quinine	-	+	-	+	-	+	-	+	-
Triterpenoid	+	-	+	-	+	-	+	-	+

In the above table preliminary phytochemical evaluation performed for plant extracts of H. antidysentrica (Roth.) Wall, Erythrina indica (Lam.) and Moringa oleifera (Lam.) presence of Phytoconstituents were obtained in their different solvent extracts.

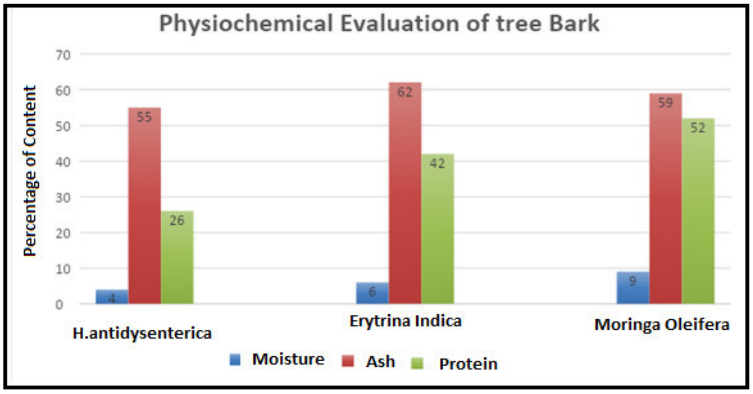
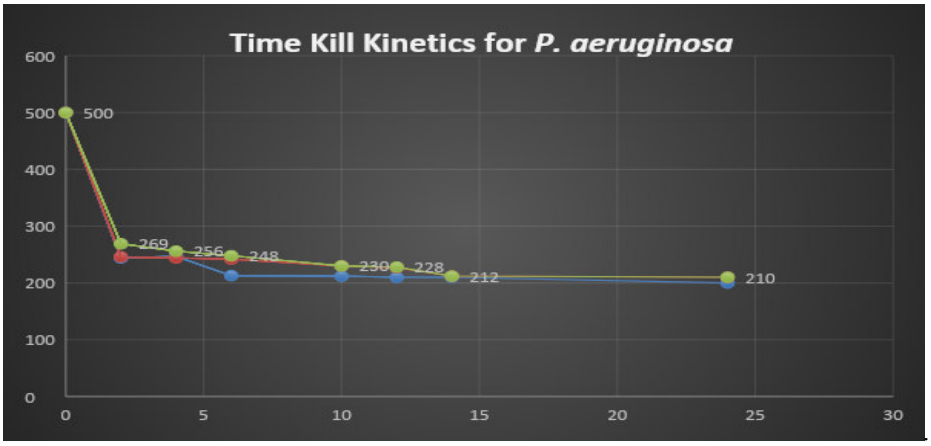
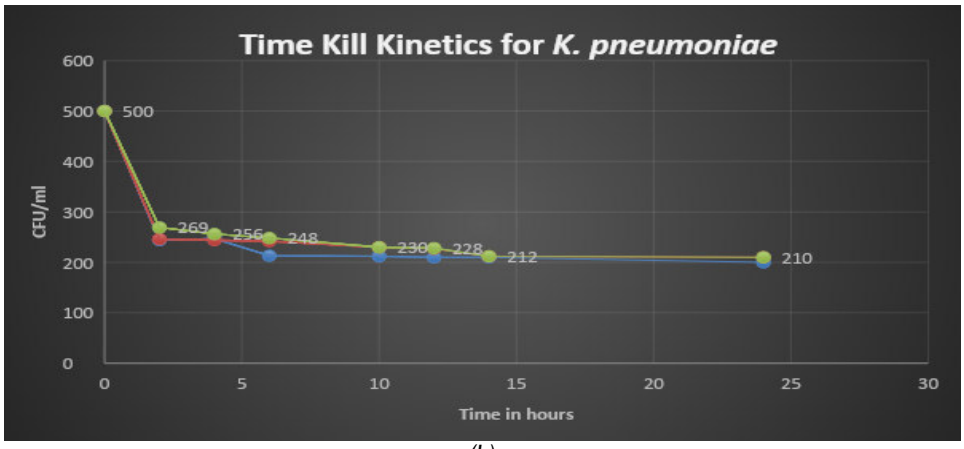


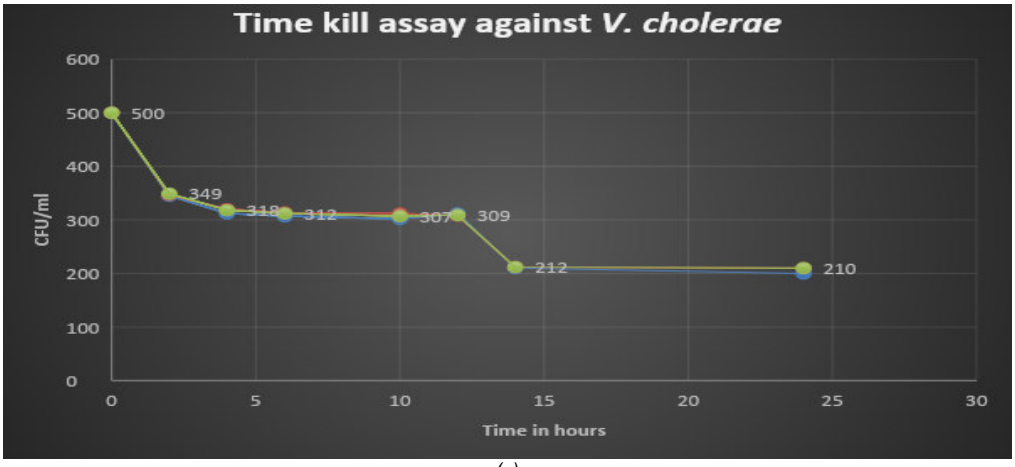
Figure 1: The above plot represents the Physicochemical evaluation of the Tree Bark, Ash content was found most in the bark of Erythrina indica Lam.; While Protein content was found to be highest in Moringa oleifera* (Lam.) *H. antidysentrica* (Roth.)Wall. *was found to be average in physicochemical Evaluation.



(a)



(b)



(c)

Figure 2 (a) , (b) and (c) Time kill Kinetics of Bark extract was represented slight fall of microbial colony when observed after the time gap 2, 4, 6, 8, 10, 12, 14, 24 hours against V. cholerae, K. pneumoniae and P. aeruginosa.

H. antidysentrica (Roth) Wall. is currently being used in the treatment of various disease conditions with out standardization. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent[25]**.** The preliminary phytochemical evaluation (Table 1) revealed the presence of several secondary metabolites which are known to possess various pharmacological effects. In last four decades the scientists are keen to evaluate many plant drugs used in medicinal folklore, due to their specific healing properties, health action and non-toxic effects.[26]In this dimension pharmacognostic study of *H. antidysentrica* is a substantial step and it further requires a long term study to evaluate pharmacological action as well as the therapeutic efficacy and toxicity of plant parts to establish as the drug. The pharmacognostic study of *H. antidy senterica* (Roth) Wall. has been carried out for the first time. This could also serve in the identification and preparation of a monograph on the plant. The plant *Moringaoleifera* family *Moringaceae* possess a broad spectrum of pharmacological activities. Also, most of the parts of plants like seeds, leaves, flowers and roots are used for treatment of various diseases. Literature reports

that aqueous, ethanolic and methanolic extracts are widely used for investigation, identification, and estimation purposes. In future the active constituents can be isolated and formulated into suitable dosage form and delivery system. Also, in future in-vivo studies based on animal models can be done for better effect[27]. In this research *Moringa oleifera* (Lam.) was found to possess several various phytochemical entities like phenol in petroleum ether extract, Steroid in ethanolic extract, protein in aqueous extract that could denote that the extraction were efficient and targeted phytochemical entities were found present. The time-kill kinetics profile of extract of bark of *H. antidysentrica* (Roth) Wall. against the test organisms *K. pneumoniae, P. aeruginosa* and *V. cholerae* at test concentrations of *Moringa Oleifera* showed reduction in number of viable cells over the first 4, 8, and 12 hours *P. aeruginosa,* and *V. cholerae,* respectively, followed by a gradual rise up to the 10th h and 14thh for *K. pneumoniae for* when compared to the control (organisms without antimicrobial agent) (Figure 2b). The time-kill kinetics profile of extract against the test organisms; at test concentrations studied showed reduction in number of viable cells over the first 2, 6, and 24 hours *K. pneumoniae, P. aeruginosa*, and *V. cholerae* respectively, followed by a gradual rise up to the 24thh for *P. aeruginosa* and *V. cholera* and 12thh (Figure 2 a,b,c) for *K. pneumoniae* when compared to the control[28].

4. CONCLUSION

It is noteworthy that these trees which are in abundant in outskirts of Hathwa region of Gopalganj (Bihar) of plants used by the practitioners of this area could reach verge of disappearance have been found to have relevant pharmacological activities, which validate their traditional usages. Among the plants used by the local Ayurveda and Unani practitioners, the plant Erythrina indica (Lam,), *Moringaoleifera*Lam. and Holarrhena antidysenterica (Roth.)Wall. merit extensive scientific researches. The complete evaluation and record completion is mandatory therefore further analytic researches such as GC-MS and HPLC could make this work implicate and effective.

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6. AUTHORS CONTRIBUTION STATEMENT

In this research work all the Laboratory work was performed by Miss Sarika Kumari Under the Supervision Dr. Sarfaraz Ahmad and also he has helped in writing work. Further the data was brought to composition as research articles. All the authors have made discussions on methodology as well as results and compiled the manuscript.

7. CONFLICT OF CONFLICT

Conflict of interest declared none

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