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Research Article

Isolation and Characterization Of Bioactive Phytochemicals

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ABSTRACT

The study of bioactive phytochemicals has gained significant attention due to their therapeutic potential and role in drug discovery. This research focuses on the isolation and characterization of bioactive compounds from selected medicinal plants. Employing advanced extraction methods such as solvent extraction, Soxhlet extraction, and maceration, the phytochemicals were efficiently isolated. Subsequent characterization was carried out using chromatographic techniques (TLC, HPLC, GC-MS) and spectroscopic methods (UV-Vis, FTIR, NMR) to determine their structure and functional groups. The results revealed the presence of key phytoconstituents such as alkaloids, flavonoids, terpenoids, phenolics, and glycosides, which are known for their pharmacological activities including antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. This study underscores the importance of natural compounds as a valuable source for new drug development and supports further exploration of medicinal plants for potential therapeutic agents.

INTRODUCTION

Plant extracts are one of the best sources of bioactive molecules, and are being used globally as an antioxidants and natural antimicrobial compounds. In current study, we have screened 40 plants against pathogen i.e. Xanthomonas oryzae pv. oryzae (Xoo), Magnaporthe grisea (MG) and X. oryzae pv. oryzicola (Xag). Based on literature review we collected 40 plants from 24 families from different region of Amarkantak which includes–Andrographics paniculata, Adhatoda vasica, Guizotia abyssinica, Bidens pilosa, Parthenium integrifolium, Vernonia anthelimntica, Agerantum houstonianum, Cosmos bipinnatus, Achyranthes aspera, Caesalpinia bonduc, Cassia tora, Cynoglossum lanceolatum, Flemingia semialata, Thalictrum foliolosum, Vitex negundo, Bauhinia retusa, Thespesia lampas, Cissampelos pariera, Xylocarpus granatum, Bryophyllum pinnatum, Lantana camara, Ocimum tenuiflorum, Mallotus philippensis, Calotropis procera, Clerodendrum

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thomsoniae, Centella asiatica, Hedychium coronarium, Bassia scoparia, Tinospora cordifolia, Datura stramonium, Murraya koenigii, Jatropha curcas, Cascuta reflexa, Urginea indica, Schefflera vinosa, Holarrhena antidysenterica, Rubia cordifolia, Argemone mexicana, Colebrookea oppositifolia and Nepeta hindostana. All plants were washed and kept at room temperature for to make it dry for extraction. Extraction results revealed that Methanol extract yield is better than other solvents extraction yields. Subsequently, additional experiments were carried out in both the solvents. The highest extraction yields were obtained in Methanol extract of Lantana camara, Holarrhena antidysenterica and Agerantum houstonianum compared to chloroform extract. Further, the qualitative determination of both Methanol extracts and chloroform extracts was performed to confirm the presence of Chloroform extract of saponin, tannin, flavonoids, steroids, anthocyanin, alkaloids, terpenoids, glycosides, Quinones, coumarins and phenols. The results obtained demonstrated the existence of various phytochemicals in both the extracts, however, most of the phytochemicals were heavily present in methanol extract. Total thirteen plants which include Bidens pilosa, Parthenium integrifolium, Vernonia anthelimntica, Agerantum houstonianum, Achyranthes aspera, Thalictrum foliolosum, Thespesia lampas, Bryophyllum pinnatum, Ocimum tenuiflorum, Tinospora cordifolia, Datura stramonium, Schlefferavinosa and Holarrhena antidysenterica were found to be endowed heavily with tested phytochemicals. After qualitative screening the antimicrobial screening was performed using methanol extracts and chloroform extracts. However, chloroform extracts have not shown activity against Xoo and M. oryzae. Hence Methanol extract was selected for antimicrobial study. The methanol extract of Agerantum houstonianum was highly effective against M. oryzae followed by Achyranthes aspera. Apart from these two mentioned extracts, methanol extracts of Thalictrum foliolosum, Schefflera vinosa, Thespesia lampas and Lantana camara have shown noticeable inhibitory activity against M. oryzae. Whereas, methanol extract of Holarrhena antidysenterica and Bidens pilosa were found to be highly effective against Xoo as well as X. oryzae pv. oryzicola. Additionally, microscopy study revealed that minimum spores were observed in plate containing Ageratum houstonianum methanol extract followed by Thespesia lampas, Thalictrum foliolosum, Achyranthes aspera, Lantana camara, Schefflera vinosa, Bidens pilosa and Holarrhena antidysenterica. Further, pathogenicity test was done which also confirmed the best results with methanol extract of Holarrhena antidysenterica against bacterial blight disease in rice. Several secondary metabolites found in plant extracts, such as Polyphenols, tannins, flavonoids, phenols, and saponin, form complexes with bacterial proteins and may impair enzymatic function, resulting in bacterial growth inhibition. The solubility of extract ingredients also determines the efficacy of extract against bacteria. The solubility of extract ingredients and the quantity of enzymes and phytochemicals could be linked to extracts enhanced antibacterial activities. Our findings clearly illustrate the efficacy of methanol extracts against Xoo and M. oryzae, paving the way for its agricultural application against rice pathogens. Quantitative phytochemical analysis was performed for eight plants based on primary observation. TPC, TFC and TAC were better in methanol extract in comparison to chloroform extract. Highest amount of TPC, TFC and TAC was observed in methanol extract of Lantana camara. Similarly, antioxidant assay (DPPH, FRAP and ABTS) further revealed that better activity of methanol extract than chloroform extract. The ability to reduce the amounts of free radicals was found best in methanol extract of the

spesia lampas. The best IC50 value for DPPH and ABTS was found in methanol extract of Thespesia lampas. The IC50 value of methanol extract was better than chloroform extract. As a result, methanol extract could be a potential free radical inhibitor, making it a good alternative for preventing chain reactions during lipid peroxidation and possibly assisting in developing nutraceuticals. Methanol extract of Lantana camara flower, Lantana camara leaf, Holarrhena antidysenterica, Bidens pilosa, Agerantum houstonianum, Achyranthes aspera and Schefflera vinosa was found significant to protect plasmid DNA (pGEM-T) against Fenton's reagent-induced oxidative damage. This study suggests the free radical scavenging capacity of the plant extracts, further signifying their application in preventing free radical mediated diseases. The Peroxidase and Polyphenoloxidase enzyme activity of Lantana camara (Leaf and flower), Agerantum houstonianum, Achyranthes aspera, Holarrhena antidysenterica, Bidens pilosa and Thalictrum foliolosum was observed high, whereas, the polyphenol oxidase was found high in Holarrhena antidysenterica and peroxidase in Ageratum houstonianum respectively. Peroxidase activity was higher than polyphenol oxidase activity in all methanol extract except Lantana camara flower, which could be due to hydroxylation occurring during polyphenol oxidase activity, which could be a potential cause for reduced polyphenol oxidase stability. Thus, the plants with high enzyme activity could be a potential source of antimicrobials and natural enzymatic antioxidants.UV-VIS and FTIR study of methanol extracts confirmed the richness of active compounds in plants. Existence of C-H, C-O, C=O, C-N, C=C, O-H, N-H functional groups were reported in many plants like Achyranthes aspera, Ageratum houstonianum, Thalictrum foliolosum, Bidens pilosa and Holarrhena antidysenterica which demonstrate the presence of various Metabolites in plant extracts. FTIR detected phenolic substances, proteins, primary and secondary amines, aromatic ethers and phenols, aldehyde and carbonyl compounds, and fatty acids, which are most likely responsible for the extracts' antibacterial effects. Since, FTIR alone could not detect all active compounds of the extract's active ingredients, GC– MS was also used. Varying number of peaks were identified in different plants like in Lantana camara (65 peaks), Thespesia lampas (16 peaks), Schefflera vinosa (61 peaks), Agerantum houstonianum (58 peaks), Achyranthes aspera (48 peaks), Holarrhena antidysenterica (64peaks), Bidens pilosa (47 peaks), and Thalictrum foliolosum (88 peaks). A few major compounds identified through GC-MS includes Kinic acid, 5- Methanol extractthyl-2(3H)- furanone, Formic acid, allyl ester, Guaiacol, 1-Oxaspiro [3.5] nona-5,8-dien-7-one, 3- methylene, 2- methoxy-4-vinylphenol, Syringol, Cytosine riboside, and 2,20 -Bioxirane etc. possess various biological functions including antioxidant, antifungal, anticancer, antibacterial, insecticidal, antidiabetic, anti-inflammatory etc. Thus, the identification of several phytochemicals revealed the therapeutic capabilities of plants. These findings may aid in a more sensible assessment of plant multipurpose use.According to a molecular docking study, few identified phytomolecules have a high affinity for bacterial and fungus receptors such as MAPK1 from M. oryzae and PDF from Xoo. In current study, Squalane from Agerantum houstonianum, Pregnane from Thalictrum foliolosum,2-Ethylhexyl methyl isophthalate from Holarrhena antidysenterica, Isoapiol from Bidens pilosa etc. have shown high binding affinity to pathogen receptors. Additionally, Ligplot and ADMET analyses demonstrated that extracts are safe to use in the field due to the low risk of undesirable environmental dispersal. As a result, due to its non-carcinogenic and low toxicity, the extract

could be safe for pathogen treatment of rice in the field with consideration of human and surrounding wildlife safety along the food chain. However, more investigation is required to isolate and identify the reliable antioxidant and antimicrobial molecules present in the crude extracts.

1) About Amarkantak:

The Amarkantak area, located in Madhya Pradesh's Anuppur district, is a rich natural heritage region that serves as the meeting place of both the Vindhya ranges and Satpura Hills, with both the Maikal Hills serving as the fulcrum. Amarkantak region lies in central India of Biosphere Reserve known as the Achanakmar-Amarkantak region. This place is rich of floristic diversity, with various plants of commercial importance. These plants are utilized by various tribes and non-tribal populations of the area in traditional indigenous therapy methods for a variety of diseases. Despite, a number of plants in the region are untouched to determine their unexplored biological activity. Almost 74% Population of Madhya Pradesh depends on agriculture either directly or indirectly. In India, rice production is basically affected by Bacterial Leaf Blight (BLB) caused by Xanthomonas oryzae pv. oryzae (Xoo) and Rice Blast caused by the fungi Magnaporthe oryzae (M. oryzae) and also many other phytopathogens are affecting the rice yield. BLB is now observed almost every year in several districts of India including Madhya Pradesh. Several diseases such as bacterial leaf streak, BLB and rice blast vastly reduce average productivity. The current rate of genetic gain and productivity through conventional plant breeding techniques, marker-assisted breeding, genetic engineering (direct alteration of species genetic materials), chemical-based approaches may not meet the food demand alone. Also, these techniques have some demerits like a time-consuming, land requirement and labor-intensive procedure, ecological side effects have been noticed, have shown harmful effects on non-targeted organisms, biosafety issues, ethical concerns and socioeconomic issues involved with the approaches. Widespread use of chemical pesticides to control pathogens cause harmful side effect on humans as well as animals. It is believed that only 0.1 percent of agrochemicals used in crop protection reach the target pathogen, eventually leaving 99.9% to harm non-target creatures in the environment. Because of their unwanted characteristics such as high as well as acute toxicity, prolonged degradation intervals, accumulation inside the food chain, and then an expansion of its power to eradicate both useful and destructive organisms, a large variety of synthetic pesticides have already been banned there in Western world. Therefore, to meet the food demands considering the harmful impacts of synthetic pesticides on life-sustaining systems, new strategies are required to meet the demand in coming years.

Status of proposed work (National and International gaps):

Many diseases caused by viruses, bacterium, and fungi impact rice productivity. Rice blast as well as BB in rice are the most destructive disease of rice. The BB disease has been known in Japan since 1884; however, its bacterial nature was established in 1992. It reduces grain yields up to 80%. Chemical substances used to treat these diseases have harmful impacts on humans and the environment. It is among the most common and destructive rice diseases in Latin America, Asia, Africa, the United States, and Australia where rice is grown in tropical climates. The devasting nature has been realized many times in India and Pakistan for long time. Japan normally witnesses the yield loss of about 20 to 23 %, but sometimes increases

very high. However, it has recently been recorded in a number of South African nations, including Mali, Burkina Faso, and Niger, in the year 2011.Given the severity of the threat, numerous measures for controlling BB have been implemented and tried, including agricultural practices, host genetic enhancement, chemical treatments, disease forecasting, and host plant genetic resistance. However, none of them seem to have been able to control the condition completely; biological control with natural products, on the other hand, has shown more significant promise. As a result, different measures to counteract Rice blast & BB are required. Moreover, 90% of the total rice cultivation is done in Asia where more than 60% of the world's population lives. To date, global rice production has been able to meet population demands. However, the continuation of this scenario in the future is a big question mark due to the world's ever-increasing population, which is expected to rise from 7.5 billion to 10 billion by 2050. To reach food security, the UN/FAO estimates that global food demand will need to rise by more than 40% until 2030 and 70% before 2050. Rice makes up a major portion of total Indian food grain production. Rice is grown in India from 8ºN to 35ºN latitude, and at elevations ranging between sea level to 3000 meters beyond mean sea level. Department of Agriculture, Cooperation and Farmers Welfare is establishing agricultural development plans to boost rice production and productivity in the country specifically, on rice, the "National Food Security Mission" (NFSM) and "Bringing Green Revolution to Eastern India" (BGREI). Rice is farmed on 43.86 million hectares in India, with a production of 104.80 million tones and productivity of around 2390 kg/ha. It may be grown in a variety of climatic and soil conditions. In comparison to many other countries, India's rice productivity is poor. Another constraint in growing rice yield in the country is that about 90% of the farmed area belongs to marginal, medium and small farmers. As a result, there is plenty of room to boost rice yield in the country. Improved technology and a variety of interventions could be implemented to boost the country's production. Hybrid rice cultivation has the potential to boost productivity and should be encouraged.

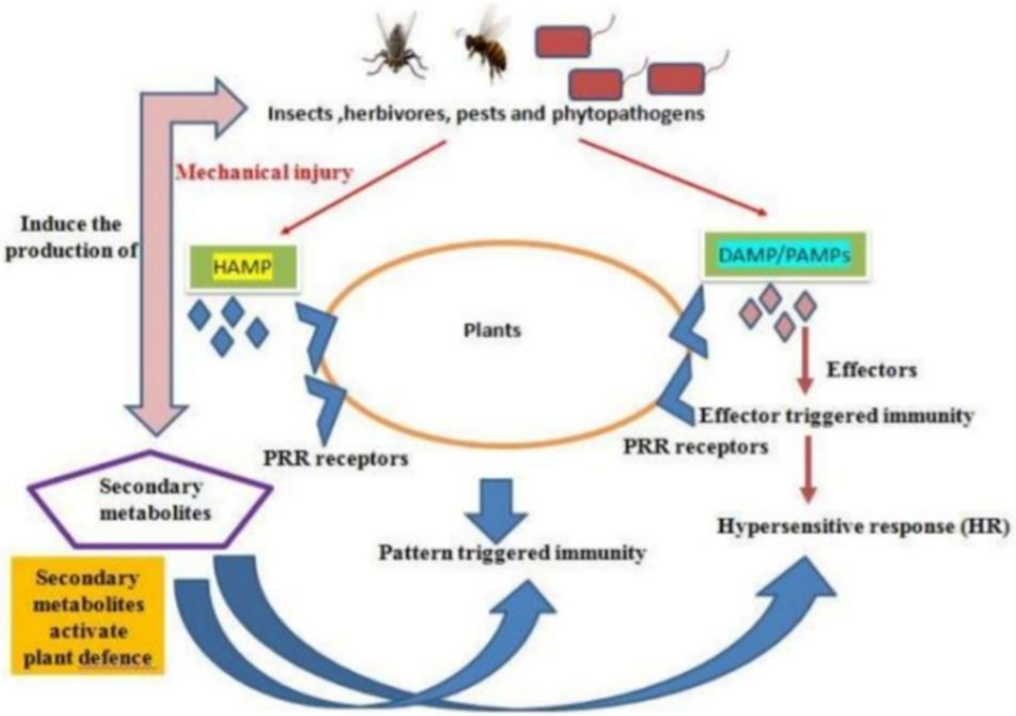
Identification research gap in the proposed field:

Bordeaux mixture, bleaching powder, zinc sulfate, tecloftalam, phenazine oxide, nickel dimethyl dithio carbamate, Agrimycin, Terramycin, Streptocycline, Brestanol, Fytolan and Vitavax were advocated as a preventive measure for BB and were reported to reduce disease incidence to some extent (Singh, R. A., et al., 1980; Devadath, S.,1989; Nasir, M., et al., 2019). Azoxystrobin, Carbendazim, Flutriafol, Moncozeb, Thiophanatemethyl, Difenoconazole + Propiconazole and Fluopyram + Tebuconazole were used as a preventive measure to control rice blast disease (Kongcharoen, N., et al., 2020). Disease forecasting and other measures were also taken into consideration, but none of them were found promising in checking the disease multiplication. However, plant products/extract have been effective against several diseases.

Scientific instruments used for sample analysis:

Analysis of the sample is the most important part of the research work. Without data analysis we cannot identify and confirm the effective compounds. Different techniques used include fluorescent microscope, UV-visible spectroscopy, Fourier Transform Infrared (FTIR)

spectroscopy, Gas chromatography mass spectroscopy (GC-MS), and Bioinformatics tools like Molecular docking as well as ADMET were used for identification of effective compounds. Few instruments used in our work are shown in the picture as UV visible spectrophotometer Shimadzu, UV-1800 (Figure 1A), Fourier Transform Infrared (FTIR) spectroscopy (Figure 1B) in IGNTU, Amarkantak. Gas chromatography-mass spectroscopy (GC-MS) of SHIMADZU, QP2010 PLUS (Figure 1C), Fluorescent microscope (Figure 1D).



Diagrammatic representation of plant defense system and production of secondary metabolites in response to attack by insects, herbivores, pests and phytopathogens.

MATERIALS AND METHODS

The current study was mostly conducted at Indira Gandhi National Tribal University (IGNTU), Amarkantak, India and few experiments was conducted at ICAR - National Institute for Plant Biotechnology, New Delhi. The materials used for the experiments and procedure followed is described below.

Collection of Plants:

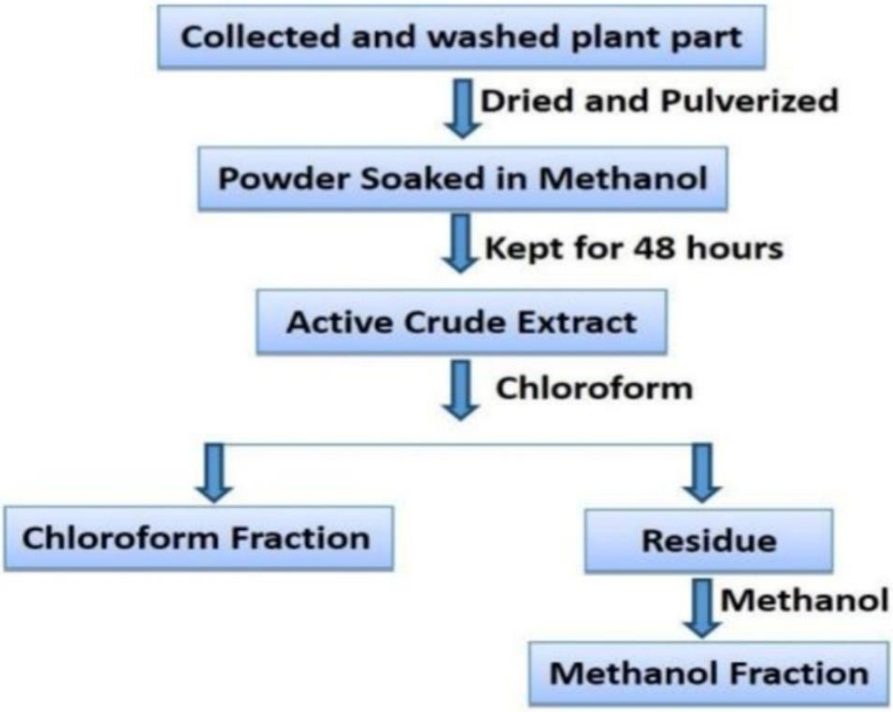
Plants of different families like Andrographics paniculata, Adhatoda vasica, Guizotia abyssinica, Bidens pilosa, Parthenium integrifolium, Vernonia anthelimntica, Agerantum houstonianum, Cosmos bipinnatus, Achyranthes aspera, Caesalpinia bonduc, Cassia tora, Cynoglossum lanceolatum, Flemingia semialata, Thalictrum foliolosum, Vitex negundo, Bauhinia retusa, Thespesia lampas, Cissampelos pariera, Xylocarpus granatum, Bryophyllum pinnatum, Lantana indica, Ocimum tenuiflorum, Mallotus philippensis, Calotropis procera, Clerodendrum thomsoniae, Centella asiatica, Hedychium coronarium, Bassia scoparia, Tinospora cordifolia, Datura stramonium, Murraya koenigii, Jatropha curcas, Cascuta reflexa, Urginea indica, Schefflera vinosa, Holarrhena antidysenterica, Rubia cordifolia, Argemone mexicana, Colebrookea oppositifolia and Nepeta hindostana were collected from Achanakmar-Amarkantak Biosphere Reserve (AABR) area of Madhya Pradesh, India, at different geographical coordinates. The plants that had been collected were authentically identified at IGNTU, Department of Botany. The plant parts were washed softly through tap water to eliminate undesirable dirt particles. Subsequently, samples were air-dried under shade and crushed to fine powder using mechanical grinder.

Reagents used:

All chemicals such as gallic acid, sodium carbonate, ammonium molybdate, sulfuric acid, sodium phosphate, ascorbic acid, potassium ferricyanide, TCA (trichloroacetic acid), quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), [2,2-azinobis (3-ethylbenzothiazoline-6- sulfonic acid) (ABTS), catechol, ferrozine, ferrous sulfate and guaiacol were purchased from Hi-Media, India. Folin-Ciocalteu reagent and Hydrogen peroxide were purchased from Central Drug House (P) Ltd. India. All the chemicals were of analytical grade. Working standards and samples were prepared with diluting stock solution into (1 mg/ml) methanol and double distilled water based on an experiment to give final concentrations. The various solvents employed in this research was of analytical quality.

Extraction and percentage yield:

determination of plant samples A few grams of plant parts are ground into fine powder using a grinder. Further, 15 grams powder was dissolved in 120 ml of 80% methanol and 95% chloroform (AR grade, 98% purity) and was placed on rotary shaker for 48 hours at room temperature (RT). The slurry obtained were centrifuged for 15 minutes at 10,000 rpm. Filtration of the supernatant were added into 50 ml volumetric flask and kept at 40˚C in water bath for solvent evaporation. The crude extract was obtained and kept in the refrigerator until further study. The dried extract was dissolved in methanol and distilled water based on desired experiment.



Qualitative- Preliminary phytochemical screening:

Plant extracts were screened for phytochemicals using the procedure described previously to unveil the presence of tannins, saponins, steroids, flavonoids, anthocyanin, and alkaloids, terpenoids, glycosides, quinones, coumarins and phenols. Below table 3 indicates the qualitative experiments performed, test name and reagent used.

Sr.No	Phytochemicals	Tests	Reagents
1.	Saponins	Foam test	-
2.	Tannins	Lead acetate Test	a 10% solution of lead acetate
3.	Flavonoids	Sodium hydroxide test	Diluted HCl and NaOH
4.	Steriods	Salkowski's Test	Conc. H_2SO_4 and Chloroform
5.	Anthocynins	Sodium hydroxide test	0.4g Sodium hydroxide
6.	Alkaloids	Tannic acid Test	Tannic acid solution
7.	Terpenoids	Salkowski's Test	Conc. H_2SO_4 and Chloroform
8.	Glycosides	Glycoside Test	10 % NaOH+3ml chloroform
9.	Quinones	Sulphuric acid	1ml conc. sulphuric acid
10.	Caumarins	Sodium hydroxide	10% NaOH
11.	Phenols	Ferric chloride test	5% a ferric chloride solution

Selected microbial strains:

Virulence strains of microorganism were purchased from national Institute for Plant Biotechnology (NIPB), Pusa Campus, New Delhi, India. Fungal strain of Magnaporthe oryzae and bacterial strain of Xanthomonas oryzae pv. oryzae (Xoo) and Xanthomonas axonopodis pv. glycines (Xag) was used to estimate the antimicrobial activities of various plants extract. All these cultures were preserved using glycerol stock and stored for longer duration at -80o C.

Media used for the screening:

Bacterial culture–Fresh culture of Xoo and Xag was prepared in Peptone sucrose media which comprises sucrose (10 g), peptone (10 g) and glutamic acid (1 g) in 1-liter double distilled water (DDW). pH of media was maintained to 7.1 ±0.2 using 1M NaOH followed by addition of agar (16 g).

Fungus culture –

Mycelial culture of M. oryzae was prepared in potato dextrose agar media (PDA) comprises potato starch (4 g), dextrose (20 g) and agar (15 g) in 1-liter DDW. Each prepared media was sterilized under autoclaved at 121°C and 15 Psi for 20 minutes. Finally, approximately 25 ml of media was poured in petri dishes.

Antimicrobial activity of extracts:

Screening for antimicrobial properties of effective extracts:

The antimicrobial activity of all methanol crude extracts (ME) was investigated and screened by the Poisoned food technique in this study. The center of PDA solid media [amended with 1ml (25 mg/ml) extract], was inoculated with a small fragment of 10-day old fungi culture. Then, the plates were incubated at RT and radial growth was measure on the 4th, 8th, 14th, days after inoculation (DAI). Percentage inhibition (PI) of mycelia growth was performed through the standard method as demonstrated by Rongai, D., et al., (2015). For antibacterial study, the center of PSA agar plates [amended with 1ml (20mg/ml extract)] was inoculated with filter paper discs loaded with bacterial suspension (105 CFU/ml). The radial bacterial growth was measured on the 4th, 8th, and 14th DAI.

The zone of inhibition was measured through the Vernier caliper method in terms of the radial growth in cm. Media altered with methanol and without alteration were served as control methanol and control, respectively.

Spore count of Magnaporthe oryzae:

In addition, effect of extract on conidia growth was detected using light microscope. A piece of mycelia was scrapped off from 72 hours old culture of M. oryzae and dissolved in deionized water followed by their filtration via cheese cloth. Finally, filtrate was diluted with 10 ml distilled water and 10 ul of suspension was subjected to Neubauer hematocytometer counting chamber. The observations were made at a magnification of 10X. All experiments were performed in triplicates.

Pathogenicity test for protective and curative activity of Holarrhena antidysentericaa

against rice BB:

To evaluate the pathogenicity test of Holarrhena antidysenterica against rice BB, Nipponbare cultivar rice leaves was sprayed with a ME of Holarrhena antidysenterica before and after the inoculation with Xoo strain. The Nipponbare cultivar rice leaves were injected only with the Xoo bacteria as a control sample, another control with Xoo bacteria in presence of water treatment, andthe one with Xoo bacteria in presence of water and DMSO (0.1%) treatment. The length of the lesion was then measured at 12 DAI in presence of 100, 190 and 200 µg/ml of ME of Holarrhena antidysenterica dissolved in water and DMSO (0.1%).

Quantitative Analysis:

Total phenolic content (TPC):

The phenolics in extracts were evaluated through spectrophotometric procedure (Singleton, V.L., et al., 1999). Reaction mixture was prepared by mixing 0.5 ml (1 mg/ml) extract in Folin-Ciocalteu's reagent (10%, 2.5 ml). Mixture was incubated for 5 min followed by addition of Na2CO3 (7.5%, 2.5 ml). Similarly, blank was concomitantly prepared using 0.5ml methanol instead of extract. After incubating samples at 45◦C for 45 min in dark, absorbance was measured at 730 nm using spectrophotometer. Experiment was done in triplicates in order to obtain mean values of samples (leaves and flower of Lantana camara, whole plant of Agerantum houstonianum, whole plant of Achyranthes aspera, whole plant of Thalictrum foliolosum, root of Thespesia lampas, whole plant of Bidens Pilosa, leaves of Schleffera vinosa and whole plant of Holarrhena antidysenterica). The gallic acid calibration curve was constructed in the range of 20–100 µg/ml. Lastly, phenolics concentration was expressed in gallic acid (GAE) equivalent terms (mg GAE/g of dw).

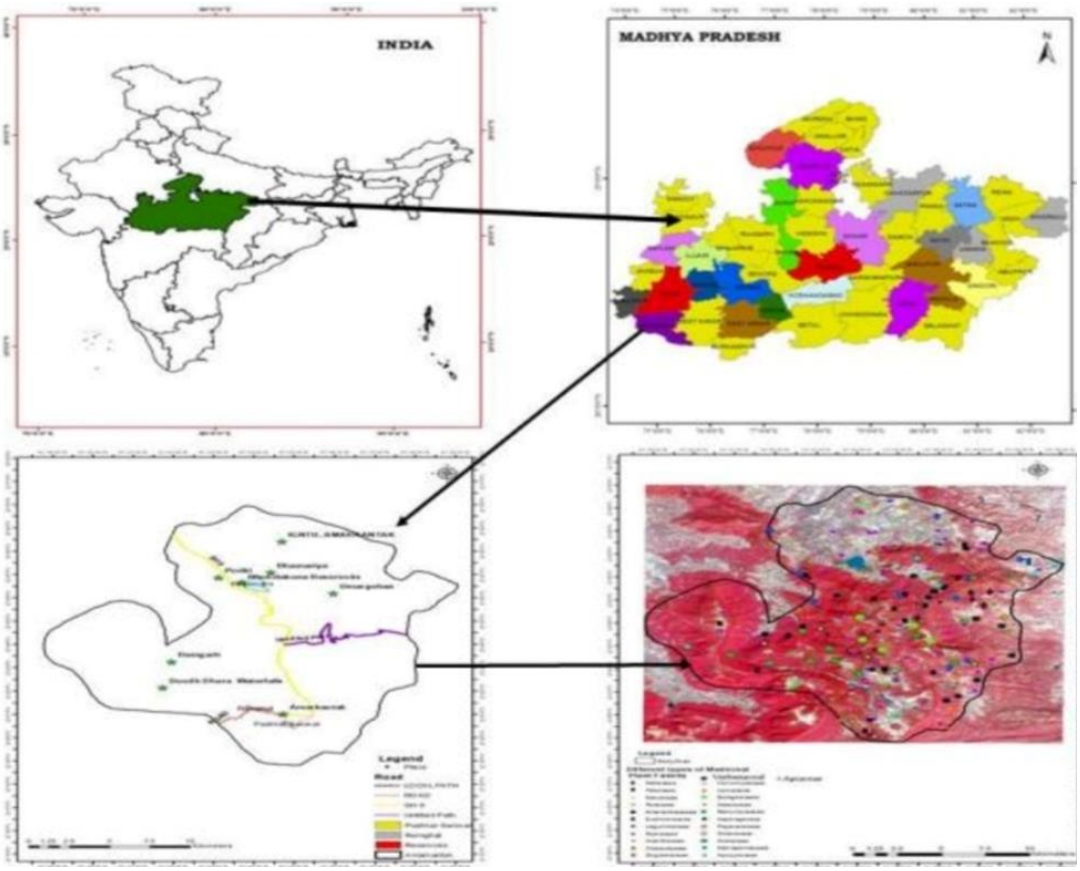
Determination of Total Flavonoids:

The total flavonoid content (TFC) of extracts is measured by AlCl3 colorimetry method. Leaves and flower of Lantana camara, whole plant of Agerantum houstonianum, whole plant of Achyranthes aspera, whole plant of Thalictrum foliolosum, root of Thespesia lampas, whole plant of Bidens Pilosa, leaves of Schleffera vinosa and whole plant of Holarrhena antidysenterica extracts were diluted with methanol till the concentration reached 1mg/ml and a calibration curve was drawn using methanol dissolved quercetin (20–100µg/ml). 2.0 ml diluted extracts/quercetin was mixed with AlCl3 [0.1ml of 10 % (w/v)], and CH3COOK (0.1ml of 0.1mM) and whole solution was prepared in methanol. Subsequently, absorbance was measured at 415 nm post 30 min of incubation at 25◦C. Finally, TFC of the extracts was represented as mg quercetin equivalent per gm dry weights (mg QE/g of dw).

Determination of Total Antioxidant (TAA):

Total antioxidant activity (TAA) of the extracts was evaluated through standard procedure with slight modification. In brief, extracts stock concentration was made to 1 mg/ml with DDW. The aliquot of 0.2 ml of every extract was mixed with 1.8 ml of reagent (0.6M H2SO4, 28mM Na3PO4, and 4mM (NH4)6Mo7O24). Then, the reaction mixture was incubated in water bath at 90◦C for 90 min, followed by cooling at RT. Samples (leaves and flower of Lantana camara, whole plant extract of Agerantum houstonianum, whole plant extract of Achyranthes aspera, whole plant extract of Thalictrum foliolosum, root of Thespesia lampas, whole plant extract of Bidens Pilosa, leaves extract of Schleffera vinosa and whole plant extract of Holarrhena antidysenterica) absorbance was read at 695 nm through UV-Visual spectrophotometer. AA was taken as standard (20–100 µg/ml) and TAA result was represented in milligram of ascorbic acid equivalents per gm extracts.

RESULTS: -



Study area and vegetation:

GIS mapping and spatial analysis of collected plants of the area:

It is feasible to create geographic databases on components of species richness and diversity using combination of spectral features as well as ground information of distribution, dominance and diversity of distinct species. A geographic distribution of all the 40 plants were depicted in single GIS- map (Fig 4 & Fig. 5) based on GPS data gathered during field survey. Such plants have also been found across hilly terrains that were difficult to reach during GPS information gathering, despite the existence of plants utilized by local peoples. All of the plants were found in abundance throughout Dindori and Anuppur Districts, with the maximum density in the Amarkantak region. To its south, the land is largely coveredby tropical moist deciduous forest, which is abundant in flora and wildlife, as well as tropical dry deciduous forest. A vast number of plant species exist there due to minimal disturbances and little anthropogenic access, and are used by the indigenous population of the area. Most of these plants flourish in urban locations, beside roads, and even in the wild and forest areas but few of them are endangered species like Holarrhena antidysenterica, Andrographics paniculata, Rubia cordifolia .

Extraction Yield (Percentage):

The use of an efficient extraction process is critical for obtaining higher yields and activities. For all 40 plants from 24 families, the percentage yield of methanol extract (ME) was higher than that of chloroform extract (CE) (Fig. 7; Table 5). The percentage yield ranged from 7.2% to 29.33% (ME) and 2.6% to 24% (CE). The top three highest percentage yield was obtained with ME of Lantana

camara (29.33%), followed by Holarrhena antidysenterica (28.66%) and Agerantum houstonianum (28%), while Vernonia anthelimntica (7.2%) had the lowest percentage yield with ME (Fig. 7 and Table 5). The top three highest percentage yield was obtained with CE is of Holarrhena antidysenterica (24%), followed by Agerantum houstonianum (22.6%) and Lantana camara (17.33%), while Achyranthes aspera (2.66%) had the lowest percentage yield in CE (Fig. 7.). The percentage yield was found in varying amounts, which could be due to polarity of the solvents which alters the solubility of the chemical present in crude extracts. In comparison to chloroform, few phenolic and flavonoid molecules form interactions with carbohydrates and proteins in methanol, making them easier to extract; this could potentially be a reason of higher yield in methanol.

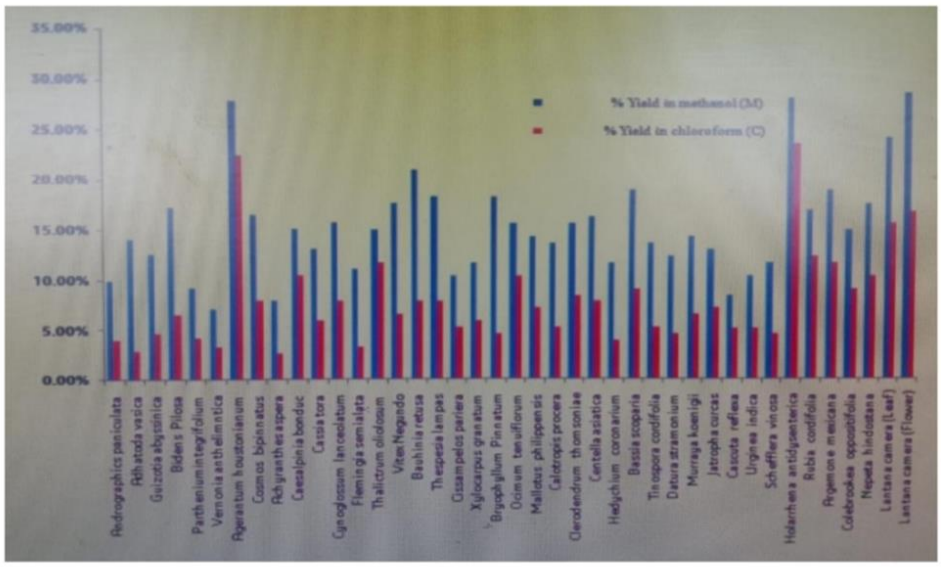
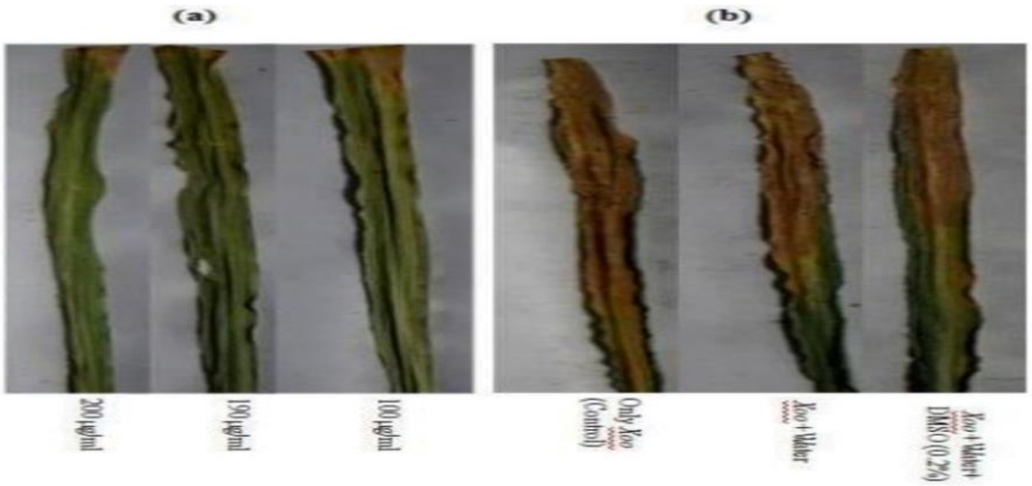


Figure 7: Percentage yield of the plant extracts in methanol and chloroform solvent

Pathogenicity test for protective and curative activity of Holarrhena antidysentericaa gainst rice Bacterial Blight.



Quantitative determination of phytochemical contents:

Tests performed	Protocol Followed	Absorbance measured at	Expressed in terms of
Total Phenolic content	Folin-Ciocalteu's reagent method (Singleton, V.L., et al., 1999)	765nm	mg GAE/ gdW
Total flavonoid content	AlCl3 colorimetry method- (Chang, C.C., et al., 2002)	415nm	mg QE/ gdW
Total antioxidant activity	(Phosphomolybdenumtest)- (Govindarajan, R., et al., 2003; Subhasree, F., et al., 2009)	695nm	mg AAE/g dW
Ferric-Reducing/ Antioxidant Power (FRAP) Assay	(Chu, Y.H., et al., 2000)	700nm	Optical Density
2,2-diphenyl-1-picrylhydrazyl (DPPH) assay	(Bursal, E., and Gulcin, I., 2011)	517nm	µg/ml
2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$) assay	(Ben, S.M., et al., 2017)	734nm	µg/ml

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