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A Comprehensive Review and Evaluation of in Vitro Antifungal Potential of Rauwolfia Serpentina

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Abstract: *Rauwolfia serpentina* L. Benth. ex Kurz. (Apocynaceae) commonly called as sarpgandha is an important medicinal plant, mainly known for its various phytochemicals. Microbial pathogens develop resistance to antibiotics after repeated administration during the treatment of infectious diseases. Therefore, it is necessary to find alternative antimicrobial drugs and the present trend is focused on medicinal plants. The hereby research work was carried out to investigate the antifungal activity of solvents as well as aqueous extracts of *Rauwolfia serpentina* whole plant, stem and roots. The main objective of present study was to evaluate a comprehensive review the antifungal activity of *Rauwolfia serpentina* L. against phytopathogenic fungi and also investigate antifungal activity potentiality of *Rauwolfia serpentina* L. The main objective of present study was to evaluate the antifungal activity of *Rauwolfia serpentina* L. against phytopathogenic fungi i.e. *Alternaria alternata*, *Aspergillus flavus* and *Mucor rouxii*. Aqueous extract of whole plant, stem and roots of *Rauwolfia serpentina* L. were prepared and antifungal activity was studied with the help of agar well diffusion assay. The aqueous root extract of *Rauwolfia serpentina* L. showed significant higher antifungal activity against *Alternaria alternata* and *Aspergillus flavus* than the other extracts under study. The present investigation clearly reveals the antifungal nature of *Rauwolfia serpentina* L. and suggests the exploitation of this plant against pest management of various plants and animal. Endophytic fungi isolates recovered from leaf, stem and root of *R. Serpentina* and a total 20 promising endophytic fungal isolates were recovered during rainy season from *R. serpentina*. Out of 20 isolates, 9 isolates were recovered from stem, 7 from leaf and 4 from root. The maximum 54% endophytic fungal strains were isolated from stem tissue, 26% from leaf tissue and 20% from root tissue of *R. serpentina*. Distribution of endophytic fungi in different tissues of *R. serpentina* had been reported in this investigation.

Keywords: *Rouwolfia serpentina*, Medicinal plant, phytopathologic fungi, in vitro antifungal potentiality, Hypertension, Herbal medicines, pharmacological activities.

I. INTRODUCTION

Rauwolfia serpentina L. Benth Kurz (Apocynaceae), commonly known as Sarpagandha, is an important medicinal plant of Indian subcontinent and South East Asian countries. It is a small, woody, perennial medicinal shrub. The International Union for the Conservation of Nature and Natural Resources (IUCN) has assigned an endangered status to *R. serpentina*. It is a medicinally famous herb in Ayurveda, Siddha, Unani and Western system of medicines (Ajayi *et al.*, 2011). It has been reported to contain 50 indole alkaloids that are mainly localized in the root. The roots are tuberous, irregularly nodular, with pale brown bark. They are of immense medicinal value and have steady demand in both domestic and international markets. In action, the root is bitter, acrid, laxative, diuretic, antidote to snake venom, expectorant and febrifuge. In folk and tribal medicine, the root of this plant is used during delivery to stimulate uterine contractions and promote the expulsion of the fetus. Crying babies are put to sleep by working mothers by making them to suck the breasts, which are smeared with the root-paste. It is also a valuable remedy in the treatment of painful affections of the bowels. Roots are used for treating various CNS disorders.

The search for new antibiotics to overcome the growing human problems of drugs resistance in microorganisms and appearance of new diseases has been rapidly increasing around the world. Realizing the capability of microorganisms to produce diverse bioactive molecules and the existence of unexplored microbial diversity, research is underway to isolate and screen microbes of diverse habitat and unique environment for discovery of novel metabolites. One such unexplored and less studied is antifungal activity of *Rauwolfia serpentina* L. From ages, medicinal plants are known to possess curative properties and hence different plant parts have been traditionally used by indigenous communities in different ways for the treatment of various diseases.

Human infections particularly those involving microorganism i.e. bacteria, fungus, viruses; they causes serious infections in tropical and subtropical countries of the world. In recent years, multiple drug resistance in human pathogenic microorganism has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases (Gutmann *et al.*, 1988 and Mohanasundari *et al.*, 2007).

Plants are the richest source of natural antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics (Mathur et al., 2011). Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens (Raghavendra, et al., 2006). Different extracts from traditional medicinal plants have been tested. Many reports have shown the effectiveness of traditional herbs against microorganisms, as a result, plants are one of the bedrocks for modern medicine to attain new principles (Evans, et al., 2002). Until natural products have been approved as new antibacterial drugs, there is an urgent need to identify novel substances active towards highly resistant pathogens (Recio, 1989 and Cragg, et al., 1997). The medicinal plants are widely used because of its easy availability and cost effectiveness. The active principles of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds (Lai and Roy, 2004). Again, Scalbert (1991) review revealed that tannins can be toxic to filamentous fungi, yeasts, and bacteria. Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity (Scalbert, 1999). Alkaloid and its derivatives have activities against *Staphylococcus aureus* and methicillin-resistant *S. aureus* (Valsaraj et al., 1997).

II. REVIEW OF LITERATURE

A. Evaluation of *in Vitro* Antifungal Potential of *rauwolfia Serpentina* (L). Benth. Ex kurz. Against Phytopathogenic Fungi

Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post harvest. In fruits and vegetables, there is a variety of fungal genera causing quality problems related to aspect, nutritional value, organoleptic characteristics, and limited shelf life (Agrios, 2004 and 2006). The most important fungi causing post harvest diseases include *Aspergillus flavus*, *Alternaria* spp. and *Rhizopus stolonifer* (Ogawa et al., 1995). Many fruits are prone to damage caused by insects, animals, early splits and during mechanical harvesting. This damage predispose the fruits to the wound invading pathogen *Aspergillus flavus* and other fungi, that causes decay on stored fruits. *Aspergillus flavus* can pose a health problem; especially it produces aflatoxin, a group of toxic, carcinogenic compound (Diener et al., 1987, Palmubo et al., 2006 and Wilson and Payne, 1994). Generally phytopathogenic fungi are controlled by synthetic fungicides, such as, thiabendazole, imazalil and sodium orthophenyl phonate (Poppe et al., 2003) however, the use of these is increasingly restricted due to harmful effects of pesticides on human health and the environment (Harris et al., 2001). Environmentally friendly plant extracts agent have shown to be great potential as an alternative to synthetic fungicide (Janisiewicz and Korsten, 2002, Zhang and Zheng, 2005). Plant extract are least expensive and cause less health hazards. Several higher plants and their constituents have shown success in plant disease control and are proved to be harmless and non phytotoxic unlike chemical fungicide. Medicinal plants were used as excellent antifungal agents because it possess a variety of chemical constituent is nature recently much attention has directed towards extracts and biologically active compounds isolated from popular plant species. In recent years, secondary plant metabolites (Phytochemicals) previously with unknown pharmacological activities have been extensively investigated as source of medical agents. Tropical plant *Rauwolfia serpentina* L. Benth ex. Kurz commonly known as sarpgandha is an important medicinal plant of Indian subcontinent and South East Asian countries (Bhatt et al., 2008, Dey and De, 2010). It has been reported to contain 50 indole alkaloids that are mainly localized in the root bark and are of great pharmaceutical interest. Reserpine is a potent alkaloid first isolated from this plant which is being widely used as an anti hypersensitive drug (anonymous, 2003). The root extract of this plant is very useful in disorder of gastrointestinal tract viz. diarrhea, dysentery and cholera and colic. So, the present work was carried out to evaluate the *in vitro* antifungal potential of *Rauwolfia serpentina* L. against *Aspergillus flavus*, *Mucor rouxii* and *Alternaria alternata*.

B. Antibacterial Activity and Preliminary Phytochemical Screening of Endophytic Fungal Extract of *Rauwolfia serpentina*

Santhosh et al., (2016) have evaluated Antibacterial Activity and Preliminary Phytochemical Screening of Endophytic Fungal Extract of *Rauwolfia serpentina* L. Benth. ex Kurz. (Apocynaceae) commonly called as sarpgandha is an important medicinal plant, mainly known for its various phytochemicals. The main objective of present study was to evaluate the antifungal activity of *Rauwolfia serpentina* L. against phytopathogenic fungi i.e. *Alternaria alternata*, *Aspergillus flavus* and *Mucor rouxii*. Aqueous extract of whole plant, stem and roots of *Rauwolfia serpentina* L. were prepared and antifungal activity was studied with the help of agar well diffusion assay. The aqueous root extract of *Rauwolfia serpentina* L. showed significant higher antifungal activity against *Alternaria alternata* and *Aspergillus flavus* than the other extracts under study. Their investigation clearly reveals the antifungal nature of *Rauwolfia serpentina* L. and suggests the exploitation of this plant against pest management of various plants and animal. Santhosh et al., (2016) have reported that Endophytic fungi isolated from *Rauwolfia serpentina*, a well known Indian medicinal plant, is used in Ayurveda for treatment of many diseases. Isolated endophytes were screened for their antibacterial activity against pathogenic bacteria. Twenty fungal isolates were recovered from different parts of the host plant and they were

characterized for their morphological features through Scanning Electron Microscopy (SEM) and on the basis of observations they were grouped in eight genus as *Fusarium sp.*, *Phomopsis sp.*, *Colletotrichum sp.*, *Cladosporium sp.*, *Aspergillus sp.*, *Xylaria sp.*, *Alternaria sp.* and *Gleomastix sp.* The secret of the fungal endophytes of this medicinal plant was revealed by the evaluation of the extract against the target bacteria. The extracts of four fungal isolates *Colletotrichum sp.* (Rs-R5), *Fusarium sp.*(Rs-R1), (Rs-R7) and *Cladosporium sp.*(Rs-S4) among twenty isolates were found effective against human pathogenic bacterial strains *E. coli* (ATCC 25922), Gram negative bacteria and *S. aureus* (ATCC 25323), Gram positive bacteria. Ethyl acetate extract of active fungal isolate (*Colletotrichum sp.*; Rs-R 5) was most effective than other extract, with maximum inhibition zone 16 mm and 14 mm and minimum MIC 25µg/ml and 36.5µg/ml against *E. coli* and *S. aureus* respectively. They Santhosh et.al.,(2016) have concluded that there are eight endophytic fungal genus isolated from *R. serpentina* as follows *Fusarium sp.*, *Alternaria sp.*, *Phomopsis sp.*, *Xylaria sp.*, *Gleomastix sp.*, *Aspergillus sp.*, *Cladosporium sp.* and *Colletotrichum sp.* Their study had shown 4 isolates out of 20 fungal isolates i.e. *Fusarium sp.* (Rs-R1, Rs-S7), *Cladosporium sp.* (Rs-R5) and *Colletotrichum sp.* (Rs-R5) had shown antibacterial activity. Inhibition zone and MIC was observed using the extract of ethyl acetate. The maximum inhibition zone (16 mm) and minimum MIC (25 µg/ml) was observed against *E. coli*. The ethyl acetate extract was found rich in secondary metabolites like alkaloids, polyphenols, flavonoids, steroids and saponins.

C. Antimicrobial Screening of Various Extracts of *Rauvolfia Serpentina*

Sharanabasappa Patil and Shruti (2015) have stated that Plant products are gaining prominence as bactericides and fungicides in view of their systemic activity and low photo-toxicity. A large number of plants are known for their antibacterial and antifungal activity, hence, they have attempted to study the antibacterial and pharmacological properties of different extracts of *Rauvolfia serpentina*. In their experimentation, they have involved Antibacterial activity of different extracts of *Rauvolfia serpentina* was assessed against E-coli, Klebsiella, Pseudomonas and S. Aureus. The following materials are used Nutrient agar medium, Sterilized Petri dishes, Pipettes of 0.1 – 0.2ml, Cultures, nutrient broth and Sterilized test tubes containing solutions of extracts of known concentration. Sharanabasappa Patil and Shruti (2015) have studied various extracts of *Rauvolfia Serpentina* for its antimicrobial and fungal properties and reported that various extracts of *Rauvolfia serpentina* fruit evaluated for antibacterial and antifungal activity. The experimental methods were used cup plate method at a dose 1 mg/ml. Among the various extracts the water and chloroform extract showed good activity against bacterial strains *E. coli* and *P. Klebsilla* and remaining Pet. ether and Ethanol extract also showed good activity against *P. aeruginosa* and *S. aureus*. The various extracts of *Rauvolfia serpentina* were studied for antifungal activity among various extracts the Pet. Ether and Ehtanol extract showd good activity against *A.flavus* and *A. niger* and remaining extracts showed poor to moderate activity. All extracts of fruit subjected to Antimicrobial screening and the results showed that the antifungal activity of plant extracts in comparison with that of standard antifungal drugs flucanazole by cupplate method the fungi selected for this were of *A. Flavus* and *A. niger*. The various extracts of *Rauvolfia serpentina* screened for antibacterial and antifungal activity. Among the various extracts the water and chloroform extract showed good activity against bacterial strains *E.coli* and *P.klebsilla* and remaining Pet. ether and Ethanol extract also showed good activity against *P. aeruginosa* and *S. Aureus*. For antifungal activity among the various extracts the Pet. Ether and Ehtanol extract showd good activity against *A.flavus* and *A. niger* and remaining extracts showed poor to moderate activity.

D. Antibacterial Activity of Isolated endophytic fungi from *rauvolfia serpentina* (L.) Benth. Ex kurz

Endophytic fungi are the most important part of biodiversity and beneficial for the survival of other organisms. Arnold, (2007); Saikkonen, et.al.,(1998) and Sibber, (2007) have reported that Endophytes play an essential role in ecological processes that includes mutualism, parasitism and commensalism. Idris et.al.,(2013) have reported that these Endophytes are symbiotic microorganisms, which live internal tissue of the plant body without causing any negative effects in the host plants. Selosse, and Schardl (2007) have reported that the reproductions in endophytic fungi take place by spores and vegetative growth showing the formation of conidia and hyphae. Arnold, (2007) had also revealed that these Endophytes are found in different parts of host plant like leaves, petioles, stem, twigs, bark, root, fruit, flower and seeds. Rodriguez, et.al., (2009) evaluated that generally, endophytic fungi are classified into different classes based on their host range, colonisation in plants and type of tissue colonised, diversity of plants, transmission and fitness benefits. Tan and Zou, (2001) and sandhu et.al.,(2014) .revealed that Endophytic fungi are produced a number of active novel bioactive compounds like alkaloids, peptides, steroids, terpenoids, phenols, quinines and flavonoids which are beneficial in agriculture, industries and in pharmaceutical industries for the production of medicine, drugs and natural biochemical's those provide protection against pathogenic organisms.

Sandhu *et al.* (2014) isolated the endophytic fungi from *Calotropis procera* (Linn.) R. Br. plant of Jabalpur region and tested their antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Salmonella typhi*, *Bacillus subtilis* and *Enterococcus* sp. The endophytic fungi *Trametes hirsute* produce podophyllotoxin and other related aryl tetralin lignans bioactive compounds which used against cancer (Zhou and Wu 2006). In other research work Kumar *et al.*, (2016) also isolated the endophytic fungi *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium solani*, *Aspergillus repens*, *Alternaria alternata*, *Alternaria* sp., *Phoma hedericola* and *Fusarium oxysporum* from *Mentha viridis* and observed their antibacterial activity against the test bacterial strain and also optimized various parameters for maximum production of antibacterial bioactive compounds.

In their experimental work Singh *et al.*, (2015) stated that endophytic fungi were isolated from the medicinal plant *Rauvolfia serpentina* also known as Sarpagandha. It is perennial woody rootstock plant commonly found all over the Indian subcontinent and South East Asian countries. More than 80 types of alkaloids are isolated from *Rauvolfia* species like reserpine. The bioactive compounds isolated from *Rauvolfia serpentina* used in a number of treatments like insomnia, hypotonic, sedative, sexual aggression, anti-hypertensive and in cardiovascular diseases. Therefore, the present study was to isolated, identified and screening of antibacterial activity of endophytic fungi isolated from different parts of *Rauvolfia serpentina*.

Rajshree Sahu *et al.*, (2016) have evaluated Antibacterial activity of isolated endophytic fungi from *rauwolfia serpentina* (L.) Benth. Ex kurz. They have isolated the endophytic fungi from medicinal plant *Rauvolfia serpentina* (L.) Benth. ex Kurz. (Family Apocynaceae) and observed their antibacterial activity against bacteria as well as the molecular characterization of most potent fungal strain. They have isolated endophytic fungi from different parts (root, shoot, leaves) of *rauwolfia serpentine* plant. Screening of endophytic fungi for antibacterial activity was scrutinised against six bacteria *viz.* *Bacillus subtilis*, *Enterococcus* sp., *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhimurium* and *Streptococcus pyogenes* by using Agar well diffusion method. For molecular sequencing of potent fungi, the DNA was extracted, quantified and amplified by using two oligonucleotide primers ITS4 and ITS6 in PCR. Their experimental results revealed that a total seven endophytic fungi *Aspergillus niger*, *Penicillium citrinum*, *Cladosporium* sp., *Curvularia lunata*, *Aspergillus* sp., *Alternaria* sp. and *Aspergillus fumigatus* were isolated from different parts of *Rauvolfia serpentina* and fungal strain *Penicillium citrinum* was shown the maximum zone of inhibition against *Bacillus subtilis* (23.0±0.12 mm), *Escherichia coli* (19.9±0.16 mm), *Streptococcus pyrogens* (19.2±0.59 mm), *Enterococcus* sp., (17.2±0.08 mm), *Klebsiella pneumoniae* (18.9±0.16 mm) and *Salmonella typhimurium* (15.1±0.16 mm). The molecular sequencing of the potent fungi was done by primers (ITS4 and ITS6) which showed strong specificity with fungal DNA and the percentages of identical matches of ITS4 and ITS6 DNA sequences in the GeneBank database (NCBI) were determined to 98 %.

They have revealed that seclusion of endophytic fungi from medicinal plant produces a number of bioactive compounds, which have a therapeutic value. Therefore, in the present work endophytic fungi were isolated from the medicinal plant *R. serpentina* and observed their antibacterial against six pathogenic bacteria. The endophytic isolated *Penicillium citrinum* was displayed the best result against all the test strains. Similar, result was observed by Sandhu *et al.* (2014) isolated the endophytic fungi from *Saraca indica* and detect their antibacterial activity against pathogenic bacteria and pointed to these endophytic fungi have the capability to produce a number of antibacterial bioactive compounds of multiple uses. Shekhawat *et al.*, (2013). were also isolated 35 endophytic fungi from leaves, the root of *Melia azadirichta* L. and observed their antibacterial activity against test strains. The endophytic fungi *Aspergillus fumigatus*, *Aspergillus japonicas*, *Aspergillus niger*, *Fusarium semitectum*, *Curvularia pallescens*, *Phoma hedericola*, *Alternaria tenuissima*, *Fusarium solani*, *Drechslera australien* and *Aspergillus repens* isolated from the medicinal plant *Ricinus communis* (L.) plant and observe their antibacterial activity against pathogenic bacteria(Sandhu *et al.*,2014). Previous research worker had examined the production of the anti-leukemic and anti-tumor drug taxol from the endophytic fungi like *Taxomyces andreanae* and *Pestalotiopsis microspora* of *Taxus* spp. (Strobel *et al.*, 1996 and Kour *et al.*,2008).

The molecular identification of *Penicillium citrinum* was also done by using oligonucleotide primer for easy identification and comparison of molecular characteristics of the fungi with other organisms. Silva *et al.*,(2011) also identified the different strain of *Aspergillus* species on the basis of their morphological as well as molecular characteristics. Nyongesa *et al.*, (2015) isolated the *Aspergillus* species from the maize kernel and soil of maize field of Nandi County and identified on the basis of macroscopic and microscopic morphological characteristics. They have concluded that the endophytic fungal strain *Penicillium citrinum* showed the potential source of antibacterial bioactive compounds and molecular sequencing of this fungus helps in future to determine the various metabolic pathways that are responsible for the production of such type of novel compounds.

Naguvanahally Somashekar Bhavana *et al.*, (2019) have worked on antioxidative and L-asparaginase potentials of fungal endophytes from *Rauvolfia densiflora* (Apocynaceae), an ethnomedicinal species of the Western Ghats and reported that the isolation and molecular characterisation of fungal endophytes from an ethnomedicinal plant, *Rauvolfia densiflora* (Apocynaceae), and an evaluation of antioxidative and L-asparaginase potentials.

Fungal endophytes were isolated from leaf and stem parts of *R. Densiflora* collected from the site of Talacauvery, Western Ghats, India. The highest colonisation frequency was found in leaves (95.5%), followed by stem parts (63.5%). A total of 19 fungal morphotypes belonging to 11 genera were identified by analysing the ITS sequences of the endophytes. The strains were screened for asparaginase production by qualitative plate assay and quantification by Nesslerisation. Maximum activity was recorded in the *Penicillium chrysogenum* culture (3.77 IU/ml). Submerged fermentation and ethyl acetate extraction were carried out to obtain secondary metabolites for the evaluation of total phenolic content (TPC) and antioxidative potentials. The TPC of fungal extracts ranged from 12.37 to 89.29 mg GAE/g dry extract and the IC₅₀ value of scavenging activity from 26.64 to 547.23 µg/ml. The *P. chrysogenum* strain (MH392736) was found to have the potentially highest total phenolic content and a high antioxidant capacity. This is the first report on the characterisation of fungal endophytes from *R. densiflora* and their antioxidative and L-asparaginase potentials. They have concluded that their study provides insight into the diversity of the endophytic fungal community isolated from *Rauwolfia densiflora*. This is the first report on the characterisation of fungal endophytes from *R. densiflora* growing in the Western Ghats. Of the 19 endophytic fungi screened for L-asparaginase and antioxidative potential, *Penicillium chrysogenum* exhibited significant results, being the relatively most frequent coloniser of leaf parts of the plant. Currently, we have initiated the secondary metabolite profiling of *P. chrysogenum* extracts to test the antiproliferative potentials in cancer cell lines. Understanding mechanisms of the antiproliferative potentials of *P. chrysogenum* may provide new insights into tumour biology, drug discovery, development of alternative drug combinations and pharmaceutical applications.

E. *Rauwolfia Serpentina* Benth Influence on Diabetes

Shah et.al., (2010) have reported that today more than 385 million people are suffering from diabetes worldwide and forecasted that 439 million adults will develop this disease in 2030 with high prevalence in developing countries. Similarly, Pakistan is facing the same problem, and it will be ranked fourth among countries with 14.5 million people having diabetes in 2025 (Shaik, et.al., 2011). Therefore, diabetes due to absolute or relative insulin deficiency or insulin resistance becomes a widespread endocrine disorder that not only affects the glucose homeostasis but also chronically alters lipid and protein metabolisms with increase in cellular oxidative stress (Rang, et.al., 2003). Commercially available pharmaceutical formulations used for the treatment of diabetes are not entirely free from side effects and do not completely restore normal glucose homeostasis (Rang, et.al., 2003). On the contrary, Tiwari, 2008 revealed that plant-based medicines are water soluble with no side effect. It has been reported that over 80%, world population is dependent on herbal medicine for their therapeutic benefits and more than 800 plant species have been mentioned in the literature with significant hypoglycemic activity (Aggrawal and Shishu, 2011). However, searching for new antidiabetic drug from natural sources including herbs is still an attractive research aspect as these are cost effective substances with no side effect. Most of herbal medicines contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, and so forth that have significant hypoglycemic effect (Pitchai et.al., 2010 and Singh, 2011). Therefore, plant kingdom has become a target for multinational drug companies and research institutes for the discovery of new biologically active compounds that could be potential antidiabetic drug with few or no side effects. Medicinally important herb *Rauwolfia serpentina* Benth (family: Apocynaceae) has an extensive spectrum of valuable therapeutic actions with mainly effective in the treatment of hypertension and psychotic disorders like schizophrenia, anxiety, insomnia, insanity, and so forth (Quraishi and Udani, 2009; Mashour, 1998). It has also reported in the treatment of skin cancers, burns, eczema, and snake bite (Harisaranraj et.al., 2009; Dey and De, 2011). Various indole alkaloids and related constituents have been isolated from the roots of this plant which have significant biological activities (Itoh, et.al., 2005). The root extract was found effective in the treatment of gastrointestinal disorders like diarrhea, dysentery, cholera, and so forth and also in breast cancer (Dey and De, 2011; Stanford et.al., 1986). An *in vitro* study described the antimicrobial and antioxidant activities of leaf extract of this plant (Dey and De, 2011).

The hypotensive action of *R. serpentina*, in terms of reserpine one of its major constituents that is commonly used as a natural tranquilizer, has been well scientifically investigated and documented (Vakil, 1955) as compared to other briefly described activities such as hypoglycaemic activity. Though, therapeutic effects of *Rauwolfia* with incomplete hypoglycemic action in diabetic patients, diabetic hypertensive patients and in anesthetized cats were brief (Cazzaroli and D. Dall'Oglio, 1958; Cohen et.al., 1959; Chatterji et.al., 1960). A preliminary study related to its hypoglycaemic and hypolipidemic activities in alloxan-induced diabetic rats has been published in 2009 by using a single dose of methanolic root extract of *R. serpentina* (Qureshi et.al., 2009) which was further elaborated by determining the acute toxicity and median lethal dose (LD₅₀) of same extract (Azmi and Qureshi 2012). Muhammad Bilal Azmi, and Shamim, A. Qureshi (2012) evaluated the phytochemistry and the effect of methanolic root extract (MREt) of *Rauwolfia serpentina* on alloxan-induced diabetic Wistar male mice.

Mice were divided in control (distilled water at 1 mL/kg) and alloxan-induced diabetic mice which subdivided into diabetic (distilled water at 1 mL/kg), negative (0.05% dimethyl sulfoxide at 1mL/kg), positive (glibenclamide at 5mg/kg) controls, and three test groups (MREt at 10, 30, and 60mg/kg). All treatments were given orally for 14 days. Qualitatively MREt showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, cardiac glycosides, phlobatannins, resins, saponins, steroids, tannins, and triterpenoids, while quantitatively extract was rich in total phenols. They have reported that flavonoids, saponins and alkaloids were also determined in root powder.

MREt found effective in improving the body weights, glucose and insulin levels, insulin/glucose ratio, glycosylated and total hemoglobin in test groups as compared to diabetic control. Similarly, significantly decreased levels of total cholesterol, triglycerides, low-density lipoprotein (LDL-c), and very low-density lipoprotein (VLDL-c) cholesterol were found in test groups. Significant lipolysis with improved glycogenesis was also found in liver tissues of all test groups. ALT levels were found normal in all groups. Thus, MREt improves the glycemic, antiatherogenic, coronary risk, and cardioprotective indices in alloxan-induced diabetic mice.

F. *Rauwolfia* in the Treatment of Hypertension

Reserpine is the most active alkaloid of *Rauwolfia serpentina* ingredient is widely studied alkaloid found in *R serpentina*. The first modern paper on reserpine was published in 1931 in the *Indian Medical Journal* by Sen and Bose cited by (Jerie,2007). It was first isolated and used by Robert Wallace Wiggins in 1950. Reserpine is one of the major alkaloids of the plant. The reserpine content has been found to be highest in the root and lower in the stems and leaves. Scientists have believed it to be the most prevalent indole alkaloid in the plant; however, different assays have challenged that assertion. The concentration of reserpine in the plant has been found to vary from 0.03% to 0.14% of the dry weight of the plant.¹⁴ The same study found that the reserpine content of the root varied from 0.038% to 0.14% in different plants. In one study, the reserpine content was 33 mg of 496 mgs of total alkaloids per gram of root.¹⁰ In another study of the *Rauwolfia* root, reserpine content was 0.955 mg/g.¹⁵ Other alkaloids in the plant have also been identified to have biochemical medicinal actions, including canescine, deserpidine, recanescine, and rescinnamine. *Rauwolfia serpentina* is a safe and effective treatment for hypertension. The plant was used by many physicians throughout India in the 1940s and then was used throughout the world in the 1950s, including in the United States and Canada. It fell out of popularity when adverse side effects, including depression and cancer, became associated with it. In 1949, Vakil (1949) reported on a study of 50 patients with essential hypertension who were treated with *Rauwolfia*. In that study, 85% of patients experienced a drop in systolic blood pressure, and 81% of patients experienced a drop in diastolic blood pressure. In 1952, Vida in Germany and Austria reported a blood pressure drop in 25 patients with hypertension. Arnold and Bach showed a good response in 37 of 50 patients in whom systolic pressure dropped an average of 30 mm Hg and diastolic pressure dropped 15 mm Hg. In 1953, Meissner reported *Rauwolfia* to be effective in 90% of a study's participants, with a lowering of systolic blood pressure between 15 and 40 mm Hg. In 1953, Loffler in Switzerland reported a lowering of blood pressure in 51 Swiss workers with hypertension. In 1954, Goto in Japan reported lower blood pressure in 12 of 15 patients with hypertension. In 1954, Doyle and Smirk in Zealand reported that reserpine produced a striking fall in blood pressure within 4 to 6 hours of administration. It has been further reported that *Rauwolfia* was the best hypertensive remedy used in India throughout the 1950s. It was reported to be used by 90% of all physicians or more than 60 000 doctors throughout the country. In a clinical trial of *R serpentina* in essential hypertension, Vakil treated 50 patients with initial blood pressures greater than 160/95 mm Hg.

Douglas Lobay (2015) has reviewed in detail *Rauwolfia* in the Treatment of Hypertension. Based on his review of the literature, *Rauwolfia* appears to be a safe and effective treatment for hypertension when used in appropriate low doses. An equivalent dose of pure *Rauwolfia* alkaloids, also known as alseroxyton extract or pure reserpine, can also be used to treat hypertension. He has found that LDR can be safely recommended to patients who have been screened to be of benefit from the treatment. The total daily dose of *Rauwolfia* should be lower than 500 mg of root and, in most cases, can be less than 250 mg per day. The dosage of purified alkaloid alseroxyton extract should be lower than 5 mg per day and, in most cases, is less than 2.5 mg per day. The reserpine dose should be lower than 500 µg per day and, in most cases, lower than 250 µg per day. An equivalent tincture dose should be based on the strength of the tincture. For instance, the dose of a 1:5 tincture would be 0.5 mL, equalling 100 mg of crude root, whereas in a standard dropper, 15 drops would equal 1.0 mL.

Ritu Soni et al., (2016) worked on The Use of *Rauwolfia serpentina* in Hypertensive Patients and reported that the root of Sarpagandha is a species of flowering plant in the family Apocynaceae has been traditionally used in Ayurveda for many years to treat a variety of diseases that at first thought appear to bear little similarity to one another. These include insanity, epilepsy, insomnia, hysteria, eclampsia and hypertension.

On reflection, however, these various diseases could have a common denominator if they were all relieved symptomatically by a sedative or a 'relaxing' drug such as Rauwolfia. In the fifty decade, its root gained popularity for its effect on hypertension. The alkaloid found in its root is attributed to anti hypertensive pharmacological action. Thus, initially serpene was isolated with an objective of predictable and better efficacy in the management of hypertension. Ayurveda believes in use of whole herb because of apparent benefits over the extract. The whole herb has many components which may be (1) Help in biotransformation into pharmacoactive forms(2) Enhance bioavailability (3) Reduce the possible side effects(4) Help in smooth excretion and(5) Prevent development of possible drug resistance.

These hypothesis is proved to be true in case of Sarpagandha as Reserpine has reported many ADRs and also human population have developed drug resistance resulting in discontinuation of Reserpine in hypertension management whereas Sarpagandha root is still in wide use.

III. MATERIALS & METHODS

A. Plant Collection

The whole plant of *Rauwolfia serpentina* L. was collected from Ranga Reddy District of Telangana State to carry out the following studies. The plant was identified at Department of Botany, Anwarul Uloom College, Mallepally, Hyderabad.

B. Sterilization of Plant Material

Whole plant leaves, flowers, stem, were kept under running tap water to remove the adhered soil particles. The plant material sterilized by addition of 2–3 drops of toluene for 3–4 minutes and washed thoroughly with distilled water 2–3 times. Surface sterilization was done with 70% alcohol for 5 minutes followed by washing with autoclaved distilled water. The plant sample was dried in the oven at 60°C to 70°C for 6 hrs.

C. Preparation of Aqueous plant extracts of *Rauwolfia serpentina* L.

Surface sterilized stem, leaves and roots of *Rauwolfia serpentina* L. were taken and crushed in sterilized pestle and mortar. The volume was made 100ml by addition of sterilized distilled water. Homogenized tissue was centrifuge at 7000rpm for 20 minutes. The supernatant was collected in another centrifuge tube and filter sterilized by Whatman filter paper No. 1 followed by storage in sterile capped bottles under refrigeration conditions (4°C) for further use.

D. Isolation of Fungi

For the purpose of antifungal evaluation, the test fungi were isolated from the rotten grapes, orange and tomato. The above fruits were kept under unrefrigerated conditions and were allowed to rot for 10 days. Rotten grapes, orange and tomato were surface sterilized using 70% alcohol for 4-5 minutes followed by washing with distilled water 2-3 minutes. The test fungus was inoculated on Sabouraud Dextrose Agar by streaking method. Identification of fungus culture was done by method of (Clark, 1981) and pure cultures were maintained at 32°C by subsequent sub culturing on SDA media (Sabouraud Dextrose Agar (Hi Media)).

E. Determination of Antifungal Activity

Antifungal activity of extract against test fungi *i.e.* *Alternaria*, *Mucor rouxii* and *Aspergillus flavus* was evaluated by agar well diffusion method (Perez *et al.*, 1990). 25µl of fungal suspension was added on the plates having SDA and was spread uniformly using a flame sterilized glass spreader. Using a sterile cork borer a well was created in the centre of the SDA Petri plates. 100µl of *Rauwolfia serpentina* L. extracts was added carefully into the well using a micropipette. Triplets were prepared for each aqueous extract against each fungus. The SDA plate with 100µl of autoclaved distilled water in the well was used as control. All the Petri plates were sealed using parafilm and incubated in an incubator at 32°C for 48 – 72 hours.

F. Determination of Minimum inhibitory concentration

After antifungal activity the plant extract that showed best result were selected and further used for determination of MIC. MIC was calculated by using different concentrations of the root extract of *Rauwolfia serpentine* L. *i.e.* 50%, 75% and 100% against most sensitive fungus. Antifungal activity of root stem and whole plant extract of *Rauwolfia serpentina* L. against test fungi *i.e.* *Alternaria alternata*, *Mucor rouxii* and *Aspergillus flavus* was evaluated by measuring inhibition zone diameter surrounding each well. Results were reported as (+) if there is inhibition of growth and (-) negative if there is no inhibition of growth.

G. Calculation of Colonising Frequency

Colonization frequency (CF%) was calculated as described by Suryanarayanan *et al.* (2003) and Photita *et al.* (200). Colonization frequency (%) of an endophyte species was equal to the number of segments colonised by a single endophyte divided by the total number of segments observed x 100.

$$\text{Colonizing Frequency \%} = \frac{\text{No. of segment colonized by fungi}}{\text{Total number of segment}} \times 100$$

H. Anti-Fungal Activity

The antifungal activity of plant extracts in comparison with that of standard antifungal drugs flucanazole by cupplate method the fungi selected for this were of *A. Flavus* and *A. niger*.

- 1) *Cup-plate Diffusion Method*: The antifungal activity of the test compounds was assessed against *A. terrus*, *A-niger*, *A-cleavatus* and *A. flamp* by plate diffusion method. The following materials are required. Nutrient agar medium, Sterilized Petri-dishes, Pipettes of 0.1ml and 0.2ml, Cultures and nutrient broth and Sterilized test tubes containing solution of test compounds at known concentration.
- 2) *Preparation of Media*: Nutrient agar medium was prepared by dissolving bacteriological peptone (5g), Beef extract (3g), Sodium chloride (3g) and agar (20g) in distill water to produce one litre of medium. Then, it was sterilized for 30 minutes at 15 lbs pressure. The pH of the solution was adjusted to 6.6-7.0 by using 40% NaOH and 6 N HCl.
- 3) *Sterilization of Media*: The media used in the present study, nutrient agar and nutrient broth were sterilized in a conical flask of suitable capacity by autoclaving at 15 lbs pressure for 20 minutes. The cork borer, Petri dishes, test tubes and pipettes were sterilized by employing hot air oven at 160°C for 1 hour.
- 4) *Preparation of Solutions of Extracts*: Solutions of all the extracts were prepared in distilled DMF and tested at the concentration 1mg/ml.
- 5) *Cup-plate Method*: This method reveals that diffusion of an antifungal from a cavity through the solidified agar layer in a Petri dish to an extent such that growth of the added microorganisms is prevented entirely in a circular area or zone around the cavity containing the solution of antifungal. A previously liquefied nutrient medium was inoculated appropriate to the assay with the requisite quantity of suspension of the microorganism and the suspension to media at the temperature between 40-50°C and immediately poured the inoculated medium into Petri dishes to give a depth of 3-4mm. It was ensured that the layers of the medium were uniform in thickness by placing the dishes on the levelled surface. The dishes thus prepared were stored in a manner so as to ensure that no significant growth or death of the test organism occurs before dishes were used and that the surface of agar layer was dry at the time of use. With the help of sterile borer, five cups of each 6mm diameter were punched and scooped out of the set agar (the cups were numbered for the particular concentration of extracts and standard). Using separate sterile pipettes 0.1ml of the prepared standard and sample solution were feel into bored cups. The dishes were left standing for 1-4 hours at room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of different solution. These were then incubated for 24 hours at room temperature. The zone of inhibition developed if any was then accurately measured.

I. Minimum Inhibitory Concentrations of Fungal Extract

The stock solution of extract of 1mg/ml concentration was prepared and by serial dilutions (10 folds) various concentrations (10-100µg/ml) were maintained to determine the minimum inhibitory concentration (MIC) values. The MIC value of endophytic fungal extracts was determined for Gram negative bacteria *E. coli* (ATCC 25922) and Gram Positive bacteria *S. aureus* (ATCC 25323). The MIC values of fungal extracts were comparable to the MIC values of standard drugs as determined by a disc diffusion method according to the CLSI guidelines (Performance standards, 2007). The concentration of both *E. coli* and *S. aureus* was 10⁶ CFU using a MacFarland standard and spread on Mueller–Hinton agar media. The disc of 6 mm was loaded with the extract and transferred on Mueller-Hinton agar medium at 37°C. MIC of the extract was determined after 48 h of incubation. The MIC value was considered as the lowest extract concentration with no visible growth.

IV. RESULTS

The antifungal activity of *Rauvolfia serpentina* (L) against phytopathogenic fungi. *Alternaria alternata*, *Mucor rouxii* and *Aspergillus flavus* are causal agent of post harvest diseases of plants as reported by Eckert and Sommer, 1967 and Adaskaveg and Sommer, 2001. Post harvest diseases account to about 50% losses in fruits stored in poor storage condition especially under high humidity. They are posing a major threat to the agriculture industry (Agrios, 2005).

On the basis of their cultural and morphological characteristics fungi identified as *Alternaria alternata*, *Mucor rouxii* and *Aspergillus flavus*. The effect of aqueous extracts of different parts of *Rauwolfia. Serpentina* are presented in Table 1 and 2. The whole plant extract, root and stem extract were effective against *Alternaria alternata* where as root and stem extract was effective against *Aspergillus flavus* and *Mucor rouxii* respectively. Root extract of *R. serpentina* recorded significant antifungal activity against *Alternaria alternate* and *Aspergillus flavus*. No positive results were reported in whole plant extract against these two tested fungi i.e. *Aspergillus flavus* and *Mucor rouxii*.

Among the three tested fungi, maximum zone of growth inhibition 15.20 mm was reported against the fungi *Alternaria alternata* by the root extract of *Rauwolfia serpentina* L. at the concentration of 100mg/ml followed by whole plant extract and stem extract.. Minimum Zone of growth inhibition found was 8.25 mm formed by whole plant extract. The adequate activity of the root extract was found against *Aspergillus flavus*. The zone of growth inhibition obtained was 6.80 mm. Several phytoconstituents like flavanoids, phenolics and polyphenols, tannins, terpenoids, sesquiterpene etc. are effective antimicrobial substances against a wide range of microorganisms. Presence of saponins and flavonoids like compounds showed the justified use of extracts from *R. serpentina* plant extract. Presence of high level of indole alkaloids i.e. reserpine, ajmaline and yohimbine in the root extract of *Rauwolfia serpentina* may be responsible for the observed antifungal activity. Cowan, 1999 reported the presence of ajmaline, reserpine and yohimbine in root extract. The antifungal activity against *Mucor rouxii* may be suspected due to presence of ajmaline and yohimbine like unknown alkaloids (Siddiqui and Siddiqui, 1931). Diameter of zone of growth inhibition was 2.75mm where as root and whole plant extract were ineffective against this pathogenic fungi.

Table 1.

Effect of aqueous extracts of root, stem and whole plant of rauwolfia Serpentina and their potentiality of inhibition of fungi

S. No.	Fungi	Root Extract	Stem Extract	Whole plant Extract
1.	<i>Alternaria alternata</i>	+	+	+
2.	<i>Aspergillus flavus</i>	+	-	-
3.	<i>Mucor rouxii</i>	-	+	-

Table 2.

Antifungal activities in terms of zone of inhibition of aqueous extracts of rauwolfia. Serpentina against tested fungi

S. No.	Fungi	Zone of inhibition in mmat concentration 100mg/ml		
		Root Extract	Stem Extract	Whole plant Extract
1.	<i>Alternaria alternata</i>	15.20	5.25	8.25
2.	<i>Aspergillus flavus</i>	6.80	0.00	0.00
3.	<i>Mucor rouxii</i>	0.00	2.75	0.00

A. Endophytic Fungi Isolates Recovered from Leaf, Stem and Root of *R. serpentina*.

Rauwolfia serpentina was selected on the basis of ethanobotanical history and their importance in Indian medicine system *Ayurveda* Pant and Joshi(2008). *Rauwolfia serpentina* has been a widespread field for study due to its various phytochemical compounds that were used in herbal medicine as a potential source of valuable drugs for the treatment of numerous diseases (Kumari et.al.,2013). A total 20 promising endophytic fungal isolates were recovered during rainy season from *R. serpentina* (Table 3.). Out of 20 isolates, 9 isolates were recovered from stem, 7 from leaf and 4 from root. The maximum 54% endophytic fungal strains were isolated from stem tissue, 26% from leaf tissue and 20% from root tissue of *R. serpentina*. Distribution of endophytic fungi in different tissues of *R. serpentina* had been reported earlier in which *Trichoderma*, *Nigrospora* and *Curvularia* were dominant (Doley and Jha (2010). The present results were in conformity with above investigators.

Table 3.
Endophytic Fungi Isolates Recovered from Leaf, Stem and Root of Rauwolfia Serpentina.

Number of Isolates				
Sample tissue from R. Serpentine				
Fungal group	Leaf	Stem	Root	Total
Fusarium sp.	1	2	1	4
Phomopsis sp.	1	–	1	2
Colletotrichum sp.	–	1	1	2
Cladosporium sp.	–	1	1	2
Aspergillus sp.	2	–	–	2
Xylaria sp.	1	1	–	2
Alternaria sp.	1	3	–	4
Gleomastix sp.	1	1	–	2
Total	7	9	4	20

V. DISCUSSION AND CONCLUSION

Rauwolfia serpentina L. Benth. ex Kurz. (Apocynaceae) commonly called as sarpagandha is an important medicinal plant, mainly known for its various phytochemicals. Microbial pathogens develop resistance to antibiotics after repeated administration during the treatment of infectious diseases. It is felt necessary to find alternative antimicrobial drugs and the present trend is focused on medicinal plants. A comprehensive review was carried out to reveal the phytochemical properties, pharmaceutical importance and mainly *Rauwolfia serpentina* plant antifungal potentiality in cure of different human ailments and diseases studied and presented in this article. In addition to this review, detailed research work was carried out to investigate the antifungal activity of solvents as well as aqueous extracts of *Rauwolfia serpentina* whole plant, stem and roots. The various extracts of *Rauwolfia serpentina* screened for antibacterial and antifungal activity. The antifungal activity of solvents as well as aqueous extracts of *Rauwolfia serpentina* whole plant, stem and roots. The main objective of present study was to evaluate a comprehensive review the antifungal activity of *Rauwolfia serpentina* L. against phytopathogenic fungi and also investigate antifungal activity potentiality of *Rauwolfia serpentina* L. The main objective of present study was to evaluate the antifungal activity of *Rauwolfia serpentina* L. against phytopathogenic fungi *i.e.* *Alternaria alternata*, *Aspergillus flavus* and *Mucor rouxii*. Aqueous extract of whole plant, stem and roots of *Rauwolfia serpentina* L. were prepared and antifungal activity was studied with the help of agar well diffusion assay. The aqueous root extract of *Rauwolfia serpentina* L. showed significant higher antifungal activity against *Alternaria alternata* and *Aspergillus flavus* than the other extracts under study. The present results were in conformity with above investigators. Distribution of endophytic fungi in different tissues of *R. serpentina* had been reported earlier in which *Trichoderma*, *Nigrospora* and *Curvularia* were dominant (Douglas Lobay, 2015; Doley and Jha (2010; Arnold, 2007; Stanford et al., 1986; and Santhosh et al., 2016). The present investigation clearly reveals the antifungal nature of *Rauwolfia serpentina* L. and suggests this medicinal plant is useful for its antifungal potentiality for curing various ailments and diseases.

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