RESEARCH PLAN PROPOSAL

Phytochemical studies of selected *Jasminum L. spp.* in Rajasthan and their Bioefficacy

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Table of contents

Content	Pg. no.
Introduction	3
Review of literature	8
Objectives of study	12
Relevance of study	13
Methodology	14
Plan of work	16
Bibliography	17
	Introduction Review of literature Objectives of study Relevance of study Methodology Plan of work

1. Introduction-

1.1. Medicinal Plants-

People have used medicinal plants throughout human history, and long before good records were kept about plant use. There is evidence that humans have been using medicinal plants not just for centuries, but for thousands of years. Otzi the "Ice-man," a 5,000-year-old man found preserved in the retreating glaciers of Europe, was carrying a pouch of mushrooms that have phytochemicals known to fight intestinal parasites, and analysis showed Otzi did indeed have intestinal parasites when he died from injuries. Texts from ancient India and China also contain descriptions of countless plant-derived medicines (Anthony A *et al.*, 2009).

Plants are the traditional source for many of the chemicals used as pharmaceuticals, biochemicals, fragrance, food colures and flavors. Most valuable phytochemicals are the products of secondary metabolism and possess sufficient chemical or structural complexity (Joshi S. G., 1990).

Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds (Edeoga *et al.*,2005).

Herbal plants are pioneer for new drug discovery and development, not only are the plant constituents used directly as therapeutic agents, but also as starting materials for synthesis of pharmacologically active compounds. The use of plants for prevention and treatment of various health ailments has been in practice since time immemorial and it is estimated that about 25% of drugs prescribed are derived from plants, moreover, WHO's essential medicine list contains 252 drugs out of which 11% is exclusively of plant origin. In the present scenario, pharmaceutical companies are involved in research on plant materials for their potential medicinal value as the demand for herbal products is growing exponentially due to its fewer side effects as compare to other system of medicines (Priya J *et al.*, 2008).

According to World Health Organization (WHO), traditional medicine is estimated to be used by up to 80% of the population of most developing countries. These plant-based medicines are used for primary health care needs (Bulletin WHO, 2002). Therefore, such plants should be investigated to better understand their properties, safety and efficacy.

Western medicine is well-known and in use, but at the same time it has created problems due to some side-effects such as carcinogenicity caused by synthetic drugs. This has enhanced the interest in search for natural products with medicinal property e.g. naturally occurring antioxidants and antibiotics for use in foods and medicine. Therefore, phytotherapy has been considered an alternative to alleviate side-effects associated with synthetic drugs (SanchezLamar *et al.*, 1999). Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available and less side effects. Indeed, the market and public demand has been so great that there is a general risk that many medicinal

plants today, face either extinction or loss of generic diversity (Mishra, 2009). Information on the chemical constituents does not only aid in discovering new therapeutic drugs, but such information can also help in disclosing new sources of economic materials such as tannins, oils, gums, that are precursors for the synthesis of complex chemical substances (Fansworth, 1996). The demand for medicinal plants is increasing in both developing and developed countries. A response to this situation is urgently needed to prevent the disappearance of plant species and the ethno-pharmacological knowledge that accompanies them (De Silva, 1997).

Within the recent years, infections have increased to a great extent and antibiotic resistance effects have become an ever-increasing therapeutic problem (Mahesh B et al. ,2008). Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Westh et al., 2004; Colombo ML et al., 1996, Iwu MW et al., 1999). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow JE, et al., 2003). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas R, et al.,2003). Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action (Ahmad I, et al., 2007; Barbour EK, et al., 2004). They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials(Iwu MW, et al., 1999) Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia N,2004).

It is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterisation of their constituents. Systematic screening of them may result in the discovery of novel active compounds (Tomoko N, *et al.*, 2002).

The use of traditional medicine is widespread in India (Jeyachandran and Mahesh,2007). A growing body of evidence indicates that secondary plant metabolites play critical roles in human health and may be nutritionally important (Hertog *et al.*, 1993). It is believed that crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects (Jana and Shekhawat, 2010). Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, steroids, glycosides and saponins etc. Secondary metabolites of plants serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Cowan, 1999).

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc (Cragg and David, 2001) i.e., any part of the plant may contain active components. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances.

India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. It exhibits a wide range in topography and

climate, which has a bearing on its vegetation and floristic composition. Moreover, the agroclimatic conditions are conducive for introducing and domesticating new exotic plant varieties (Martins AP, *et al.*,2001).

In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju AV, *et al.*,2005). **Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections** (Balandrin MF, *et al.*,1985). Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments (Tanaka H, *et al.*,2002). Various medicinal properties have been attributed to natural herbs.Medicinal plants constitute the main source of new pharmaceuticals and health care products (Ivanova *et al.*, 2005). The history of plants being used for medicinal purpose is probably as old as the history of man kind. The use of medicinal plants in the industrialized socities has been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicine (Shrikumar and ravi, 2007).

The aim of the present study was to investigate all the phytochemical compounds in selected spp. of *Jasminum* plants in Rajasthan and test their anti-microbial activity against selected bacterial strains.

Jasmines are an important group of flowering plants. They are widely cultivated and esteemed for there attractive fragrant flowers .This genus belongs to the family Oleaceae. Moreover, different parts of the plant such as the leaf, stem, bark, and roots are very useful and important in pharmaceutical industries. (Priya Joy *et al.*, 2008)

Jasminum grandiflorum Linn (Spanish jasmine, Common jasmine, Chameli, Jati)



Jasminum grandiflorum is a scrambling sub erect twining evergreen shrub [Anonymous. The Wealth of India A Dictionary of Indian Raw Materials and Industrial Products,2004, Medicinal Plants of India, 1987], native to India, France, Italy, China, Japan, Morocco and Egypt[Chopra R.N., *et al.*,1958, Chopra R.N., *et al.* 2002, Kirtikar K.R., *et al.*1989, . Sharma P.C., *et al.*,2005). The leaves are opposite, entire ovate to somewhat elliptic in shape with acuminate mucronate apex, whereas flowers are terminal and axillary cymes, calyx lobes are long, linear (Cooke T.,1967; Nadkarni A.K. *et al.*,1976). Jasminum grandiflorum has diuretic and emmenagogue properties. The fresh juice of leaves are applied to corns, and the leaves are chewed and used in the treatment of ulcerations of the mouth .The leaves contain resin, salicylic acid, and an alkaloid named jasminol. Roots are useful in cephalalgia, mental debility, chronic constipation, flatulence, strangury, sterility, dysmenorrhoea, amenorrhoea, ringworm, leprosy, skin diseases and giddiness. Leaves are useful in odontalgia, fixing loose teeth, ulcerative stomatitis, leprosy,

skin diseases, dysmenorrhoea, ulcers, wound, corns and flowers are useful in stomatopathy, cephalopathy, ophthalmopathy, leprosy, skin diseases, pruritis, strangury, dysmenorrhoea, ulcers, as refrigerant, ophthalmic and vitiated conditions of pitta (Warrier P.K.,2004). *Jasminum sambac* (Arabian jasmine, Indian jasmine, Sampaguita, Mogra)



Jasminum sambac is a member of Oleaceae family, known as sampaguita in the Philippines, where it is national flower, mogra in India, moli in China, pikake in Hawaii and Arabian jasmine in the mainland USA. It is commercially grown in India, Thailand, China and Philippines . It is an evergreen vine or shrub reaching up to 1-3 m . The leaves are ovate; phyllotaxy is opposite or in whorls of three. The flowers blooms throughout the year and are produced in clusters of 3-12 together. They are strongly scented and open at night, close in morning . *Jasminum sambac* is used for the treatment of skin diseases, ulcers and fever. The plant traditionally used as an analgesic, antidepressant, antiinflammatory, antiseptic, aphrodisiac, sedative (source :wikipedia). Roots are used to treat wounds and snake bites. The leaves are used for treatment of diarrhoea, abdominal pain, conjuctivitis and dermatitis. The leaves and roots are used for treating diarrhoea, fever, pain and as an anaesthetic (Kunming Institute of Botany ,1986 ;Jiangsu New Medical College ,1977).

Jasminum auriculatum (Needle flower jasmine, Juhi, Juyi)



Jasminum auriculatum Vahl (Oleaceae) commonly known as Juhi, Needle flower jasmine, Yutika, grows almost throughout South India, on dry slopes of the Western Ghats (Vaidyaratnam P.S.,2003). Flowers are white, sweet scented and trifoliate with two lower leaflets broadly ovate, acuminate or rounded. The roots are useful in skin diseases especially for ringworm and flowers

are fragrant, sweet, refrigerant, astringent, cardiotonic, diuretic and depurative in nature. They are useful in burning sensation, ulcers, stomatopathy, cardiopathy, nephrolithiasis and dermatopathy (Ghosh MN, 1984). *Jasminum auriculatum* leaves has been reported to contain lupeol and jasminol (Deshpande SM, *et al.*,1967). Alcoholic and aqueous extracts of flowers of *Jasminum auriculatum* showed diuretic activity by increasing the total volume of urine and concentrations of potassium and sodium salts in urine (Bahuguna Y, *et al.*,2009) and antiurolithiatic activity by reducing the elevated urinary oxalate synthesis (Bahuguna Y.,*et al.*,2009).

2. Review of literature-

Aromatic plants had been used since ancient times for their preservative and medicinal properties, and to impart aroma and flavor to food. Hippocrates, sometimes referred to as the 'father of medicine', prescribed perfume fumigations. The pharmaceutical properties of aromatic plants are partially attributed to essential oils. The term 'essential oil' was used for the first time

in the 16th century by Paracelsus von Hohenheim, who named the effective component of a drug, 'Quinta essential' (Guenther, 1950). By the middle of the 20th century, the role of essential oils had been reduced almost entirely to use in perfumes, cosmetics and food flavorings, while their use in pharmaceutical preparations had declined. Essential oils are natural, complex, multi-component systems composed mainly of terpenes in addition to some other non-terpene components. Several techniques can be used to extract essential oils from different parts of the aromatic plant, including water or steam distillation, solvent extraction, expression under pressure, supercritical fluid and subcritical water extractions.(Amr E. Edris, 2007).

2.1. Medicinal plants-

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Shariff, 2001).Antibiotic resistance has become a global concern (Westh *et al.*, 2004).

The success of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains. Methanol extracts of six plant species traditionally used in Indian folklore medicine for the treatment of various bacterial and fungal infections were investigated for *in vitro* antimicrobial activity against pathogens namely *Staphylococcus aureus*, *Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger* by disc diffusion method. Methanol extracts of *Eugenia jambolana* (Java plum, jambolan, jamun) and *Cassia auriculata* showed the highest toxicity against all the bacteria. The plant extracts showed antibacterial activity but not antifungal activity against any of the fungi used. Minimum inhibitory concentration (MIC) assay were determined for these two extracts against bacteria. *E. jambolana* revealed the highest antimicrobial activity at a minimum concentration (0.75 mg/ml) against *S. aureus*. The phytochemical analysis carried out revealed the presence of coumarins, flavanoids, glycosides, phenols, tannins, saponins and steroids. Alkaloids were not detected from any of the plant extracts under study. The results provide justification for the use of the plants in folk medicine to treat various infectious diseases (Mohamed Sham Shihabudeen *et. al.*,2010).

The antibacterial effect of some selected Indian medicinal plants was evaluated on bacterial strains like *Bacillus cereus* ATCC11778, *Staphylococcus aureus* ATCC25923, *Enterobacter aerogenes* ATCC13048, *Escherichia coli* ATCC25922 and *Klebsiella pneumoniae* NCIM2719. The solvents used for the extraction of plants were water and methanol. The in vitro antibacterial activity was performed by agar disc diffusion and agar well diffusion method. The most susceptible Gram-positive bacteria was *B. cereus*, while the most susceptible Gram-negative bacteria was *K. pneumoniae*. The extracts of *Abrus precatorius*, *Cardiospermum halicacabum*

(Small Balloon Vine) and *Gmelina asiatica* could not inhibit any of the bacterial strains investigated. The most active antibacterial plant was *Caesalpinia pulcherrima*. The significant antibacterial activity of active extracts was compared with the standard antimicrobics, piperacillin (100 μ g/disc) and gentamicin (10 μ g/disc). The results obtained in the present study suggest that Caesalpinia pulcherrima can be used in treating diseases caused by the test organisms.(Jigna parekh *et al.*,2007).

The aim of the study was to find out the bioactive chemical constituents and to evaluate the antimicrobial activity of the ethanolic extract of traditionally used eight medicinal plants of Nepal. A qualitative phytochemical analysis was performed for the detection of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugar. The highest yield of ethanolic extract was found in *Azadiracta indica* (29.08%). *Ocimum sanctum* contained all the chemicals except flavonoids and reducing sugar however the *Colquhounia coccinea* (Himalayan Mint Shrub) lacked alkaloids and reducing sugar. The antimicrobial activities of these plants extract were also observed. The extract of *Rhododendron setosum* and the essential oil of *Eucalyptus globulus* were most effective against *Escherichia coli* and *Staphylococcus aureus* respectively. But the extracts of *Azadiracta indica* and *Elsholtzia fructicosa* were found to be most effective against *Klebsiella* species. Thus, it can be concluded that use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization. Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. (Himal Paudel Chhetri *et al.*,2008).

The leaf and root extracts of Senna italica (Neutral Henna) were screened for phytochemical properties and antibacterial activity using standard methods. Alkaloids, steroids and flavonoids were detected in aqueous-methanol, n-hexane and aqueous extracts while tannins, glycosides and saponins were not detected in all the extracts. Sensitivity testing of the extracts showed a strong activity against all the test bacteria (Staphylococcus aureus, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa and Streptococcus pneumoniae), which increased with increasing concentration of the extracts (30, 60, 90 and 120 mg/ml). Thus, inhibition zones of 20.0 ± 0.82 , 32.0 ± 0.50 , 32.0 ± 0.50 , 33.0 ± 1.64 and 31.25 ± 0.25 mm were recorded at the highest concentration of 120 mg/ml for the leaf extracts against S. aureus, S. typhi, E. coli, P. aeruginosa and S. pneumoniae respectively. Similar trend was observed for the root extracts. Therefore, on the ground of the pronounced activity of the extracts against the test bacteria as well as the presence of alkaloids, flavonoids and steroids in the extracts, it could be suggested that this plant has a potential as a source of therapeutic agents. This supports the traditional use of the plant in curing human diseases. It is therefore suggested that further studies be carried out using different solvents as well as to isolate, purify and identify the active compounds present in the extracts with a view to justifying these claims.(Y.U. Dabali et al., 2012).

The aqueous and methanol extracts of 12 plants each belonging to different families were evaluated for antibacterial activity against medically important bacteria viz. *B. cereus ATCC11778*, *S. epidermidids ATCC12228*, *E. aerogenes ATCC13048*, *P. vulgaris NCTC 8313*, *S. typhimurium ATCC 23564*. The in vitro antibacterial activity was performed by agar disc diffusion and agar well diffusion method. The aqueous extracts were inactive but methanol extracts showed some degree of antibacterial activity against the tested bacterial strains. *S. typhimurium* was the most resistant bacteria while *B. cereus* was the most susceptible bacteria. Amongst the plant species screened, methanol extract of *Bauhinia variegata* bark showed best antibacterial activity (Jigna Parekh, and Sumitra Chanda ,2007).

2.2. Jasminum spp.-

In the recent years, the antimicrobial and antioxidant actions have received much attention. This is so because of the increasing interest in human health and have been studied *in vitro* and *in vivo* by many researchers. The antioxidant may be useful in retarding oxidative deterioration of food materials especially those with high lipid content. The natural antimicrobial agents protect

living organisms from damages resulting in the prevention of various diseases. There is a growing interest in substances exhibiting antimicrobial and antioxidant properties that are supplied to human and animal organisms as food components or as specific pharmaceutics (Azuma, 1995). Although, much work has been done on the antimicrobial and antioxidant effects of different plants species. Plants are the primary sources of naturally occurring antioxidants for humans. It has been well known that essential oils and plant extracts have antimicrobial and antioxidant effects (Özer, 2007).

The traditional use of this plant suggests analgesic, antidepressant, anti-inflammatory, antiseptic, aphrodisiac & sedative effects. Essential oil of J. sambac is used as fragrance for skin care products. Jasmine oil and absolute reduce skin inflammation, tones the skin and lifts up your mood. The present work reports results of a detailed analysis of antioxidant and antibacterial activities of the essential oils and methanol extracts of J. sambac contributing to the search for beneficial uses of this plant. The essential oil was subjected to screening for their possible antioxidant activity by two complementary test systems, namely DPPH free radical scavenging and Beta-carotene-linoleic acid assays. Butylated hydroxytoluene (BHT) was used as positive control in both test systems. In the DPPH test system, the IC50 value of essential oil and methanol extract were respectively 7.43 and 2.30 µg/ml. In the Beta -carotene-linoleic acid system, oxidation was effectively inhibited by Jasminum sambac, the RAA value of essential oil and methanol extract were respectively 96.6 and 93.9%. When compared to BHT, the oil and methanol extract were nearly the same value. Furthermore, the essential oil and methanol extract were evaluated for its antimicrobial activity using disc diffusion and micro dilution methods. The essential oil and methanol extract showed better activity against bacterial species than against yeast.(Fatouma Abdoul-Latif et al. ,2010).

The ethanol extract of the dried leaves of *Jasminum sambac* (L.) Aiton (Family - Oleaceae) was investigated for its possible analgesic and cytotoxic activities in animal models. The extract produced significant (P<0.001) writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 250 and 500 mg/kg of body weight comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. The crude ethanolic extract also produced the most prominent cytotoxic activity against brine shrimp *Artemia salina* (LC50 = 50 µg/ml and LC90 = 100 µg/ml). The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.(Md. Atiqur Rahman *et al.*, 2011).

Jasminum auriculatum is a shrub used in traditional medicines, Ayurveda, Siddha and Unani . Studies conducted on it show that it possess beneficial effects as aphrodisiac, antiseptic, anthelmintic, aromatherapy, cardiotonic, diuretic, hyperdipsia, leprosy, suppurative, skin diseases, thermogenic, ulcers and wounds . The present review highlights the various folk, ayurvedic uses, pharmacognostical, phytochemical and pharmacological studies conducted on *J. auriculatum* and also the unexplored potential of the plant. (Arun mittal *et al.*,2011).

Jasminum grandiflorum has diuretic and emmenagogue properties. The fresh juice of leaves are applied to corns, and the leaves are chewed and used in the treatment of ulcerations of the mouth .The leaves contain resin, salicylic acid, and an alkaloid named jasmine. Jasminum sambac is used for the treatment of skin diseases, ulcers and fever. This preliminary phyotochemical study of the antimicrobial activity of ethanolic callus extracts focused on two species of Jasminum, J. grandiflorum and J. sambac. The plants are widely used as traditional

medicine in India for skin disorders. Preliminary phytochemical analyses of the callus extracts reveled the presence of alkaloids, glycoside, flavanoid, terpines, tannin, resin, and salicylic acid. The extracts were subjected for screening of *in-vitro* antimicrobial activity against selected disease causing pathogens, *viz., Staphylococcus albus, Proteus mirabilis, Salmonella typhii*, etc., at concentrations of 500mg/ml, 250mg/ml.The results of antimicrobial activity revealed that all the extracts showed comparatively better activity than other extracts. They can be use as antibiotics (Priya Joy *et al.,* 2008).

Jasminum sambac Linn (family: Oleaceae) is commonly known as "mogra". The Plant is considered as cool and sweet; it is used as remedy in case of insanity, weakness of sight and affections of mouth. *J.sambac* flowers contain major phytoconstituents as glycosides, saponins, flavonoids and terpenoids. In this study, the phytochemical screening and anti-lipid peroxidation effect of *J.sambac* was evaluated using the standard antioxidants BHT, Vitamin C, Vitamin E and Rutin. The preliminary study shows the presence of alkaloids, flavonoids, terpenoids, carbohydrates, proteins, phenols, tannins, saponins and phytosterols. The methanolic extract of the *J.sambac* flowers shows anti-lipid peroxidative effect which is similar to that of all standards. Results of this study suggests that the methanolic extract of *J.sambac* can be used as therapeutic agents to treat against various diseases caused by free radicals and other chemical agents. (Kalaiselvi M *et al.*,2011).

The use of herbal drugs for prevention and treatment of various health ailments has been in practice since time immemorial. Literature revealed that about 25% of drugs prescribed worldwide are of plants origin. Traditionally *Jasminum* species has been used to treat dysmenorrhoea, amenorrhoea, ringworm, leprosy, skin diseases and also as ananalgesic, antidepressant, anti-inflammatory, antiseptic, aphrodisiac, sedative, expectorant therefore an attempt has been made to enumerate some of *Jasminum* species used for the alleviation of ailments. In the present article an endeavour has been made to present a potential of *Jasminum* species used for general healthcare. (Akash Jain *et al.*,2011)

3. Objectives of study-

This research was aimed at identifying and analyzing the phytochemicals present in *Jasminum spp.*, and evaluation of some biological properties of the plant with the following specific objectives:

- i) Sampling of the plants (from different- 2 locations) and prepare their extract in various reagents according to their polarity from non-polar to polar solvents.
 - ii) To carry out qualitative and quantitative estimation of phytochemicals through Physicochemical analysis.
- iii) Isolation, Purification and Identification of bioactive compounds through Thin layer Chromatography & Column Chromatography and individual characterization of each component.
- Study the anti-bacterial effect of crude extract as well as the isolated individual compound against selected bacterial strain(By Disc Diffusion method or MIC or Agar well Diffusion method) for biological evaluation.

4. Relevance of study-

Infections caused by bacteria and fungi account for approximately one-half of all deaths in the developing countries. Prevention of the microbial infections includes vaccination and development of anti-microbial agents. Although anti-microbial drugs have saved many lives, the high production cost of these drugs development of resistance in the responsible pathogens are challenges facing the healthcare system. Developing countries have the potential of plant resources as hat exhibit a wide range of biological activities which may help in the development of cheaper and more potent anti-microbial agents. Many plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant. Traditional medicine like orthodox medicine has its own methods and techniques of application which however aims at healing disease. Nearly, all culture and civilizations from ancient times to the present day have depended fully or partially on herbal medicine because of their effectiveness, affordability, availability, low toxicity and acceptability. Therefore, newer plants should be investigated to better understand their properties, safety and efficacy.

In this view, The abuse of drugs for ailment is in high increase which leads to increase the side effects & some time death so it is therefore very important to search for effective but low cost and reliable traditional therapeutic agents which helps to motivate the people to use these drugs as a resistant against diseased organisms. This work is therefore aimed at studying few spp. of *Jasminum* plants for their phytochemical properties and antimicrobial activities in comparison to known antibiotics.

5. Methodology-

- **5.1**. Identification and authentication of plant, *Jasminum Spp*. by the Curator (Herbarium, Department of Botany), University of Rajasthan, Jaipur.
- **5.2.** Samples will be collected from various locations, shade drying and transported to the laboratory in accordance with standard procedures.

5.2.1. Stem, leaves & flowers will be selected as a sample from *J. grandiflorum*, *J. auriculatum* & *J. sambac*.

5.2.2. Powdering of shade dried plant material for extraction

5.3. Hot sohxlet extraction followed by Physico-chemical analysis test

5.3.1. Qualitative estimation

• Tests for carbohydrates

(By Molisch's test, Fehling's test, Benedict's test)

• Tests for glycosides

(By Legal's test, Borntrager's test)

Tests for alkaloids

(By Mayer's test, Dragendroff's test, Hager's test, Wagner's test)

• Tests for Phyto steroids

(By Salkowski test, Liebermann- Burchard test)

• Tests for flavanoids

(By Shinoda test, Ferric chloride test, Alkaline reagent test)

• Tests for saponins

(By foam test)

• Tests for Proteins and amino acids

(By Biuret test, Million's test, Xanthoprotein test, Ninhydrin test)

• Tests for Tannins and Phenolic compounds

(By 5 % Fecl₃ sol., Lead acetate sol., Acetic acid sol., Dilute iodine sol., Dilute HNO₃)

• Tests for fixed oils and fats

(By Spot test, Saponification test)

• Tests for Gums and mucilages

(By Ruthenium test)

- Tests for Anthraquinones
- Tests for Anthocynosides
- **5.3.2.** Quantitative estimation

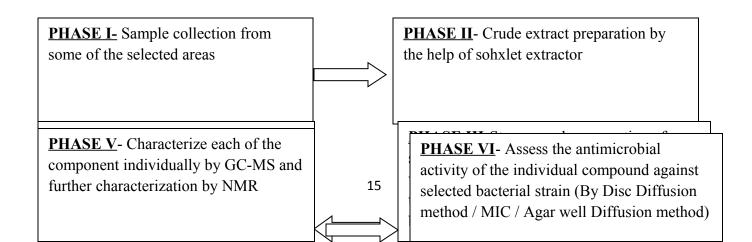
(Estimated content is calculated as % of the starting materials)

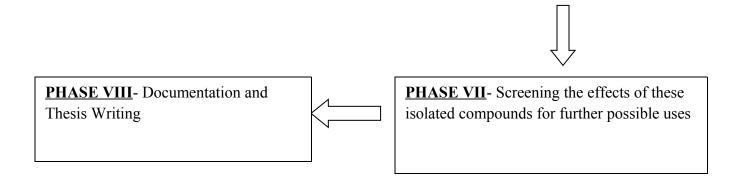
5.3.3. Extracted crude samples will be redissolve in respective solvents & preserved as mother sample for further microbial analysis through anti-bacterial activity.

5.4. Isolation

- 5.4.1. Samples collected will be processed further for Isolation, Purification and Identification of unknown compounds using Thin layer Chromatography & Column Chromatography.
- **5.4.2.** Characterization each of the component individually by some standard techniques by GC-MS & NMR.
- 5.5. Purified extract will be treated against selected bacterial strains (by Disc Diffusion method
 / MIC / Agar well Diffusion method) for further assessment of antimicrobial activity & evolution of the various biochemical parameters.

6. Plan of Work-





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