

IN-VITRO ANTI BACTERIAL ACTIVITY DETERMINATION OF IN TAMARINDUS INDICA LINN

D.Prathyusha¹, V.Kavitha², M.Srujana³, K.Venkata Gopaiah^{4*}

1,2,3. Assistant Professor, 4. Associate Professor

1,3,4. Department of Pharmaceutics, 2, Department of Pharmacology

1,2,3. Vision College of Pharmaceutical Sciences & Research-Boduppall, Ghatkesar (M), Ranga Reddy (Dist.)

4. St. Mary's College of Pharmacy, St. Mary's Group of Institutions Guntur-Chebrolu (M&V), Guntur-522212-A.P

ABSTRACT:-

Our study showed that there is great variation in fatty acids, elemental composition and total protein in *T. Indica*. Moreover As, Pb, Cd were not detected by previous works and in recent investigation 32 fatty acids were isolated from same plant. The total protein analysis of *T. Indica* medicinal plants from Jamshoro Distt. (Sindh) 15.6%. The great variation of fatty acids, elements and total protein is due to the environmental and ecological factors.

Present study revealed that *Tamarindus Indica* possesses antidiabetic and hepatoprotective activity in alloxan induced diabetic rats. From the result, it is concluded that the juice of *Tamarindus Indica* Linn leaves showed significant anthelmintic activity. when compared with the standard anthelmintic drug. The drug may be further explored for its phytochemical profile to identify the active constituent responsible for anthelmintic activity. In conclusion, our findings show that chloroform leaf of *tamarindus Indica* extract reduction on blood glucose may be due to several flavonoids, glycosides present.

Key Words:- *Tamarindus Indica*, antidiabetic, hepatoprotective activity.

Introduction :-

Tamarindus Indica L. or tamarind, as it is commonly known, is a medium-sized tree belonging to the *Caesalpinaceae* family. Tamarind has been used for centuries as a medicinal plant; its fruits are the most valuable part which have often been reported as curative in several pharmacopoeias. Nevertheless, other plant parts have been less studied. The leaves have a proven hepatoprotective activity associated with the presence of polyhydroxylated compounds, with many of them of a flavonolic nature.^[1] The seeds and the bark also have medicinal properties.

Due to their antimicrobial, antifungal and antiseptic effects, tamarind leaves have an extensive ethnobotanical use in many areas of Latin America such as Mexico, Puerto Rico, and Trinidad and Tobago, and in other continents like Asia and Africa within the multi-ethnic Cuban population – very closely related to other Caribbean countries such as Puerto Rico, Trinidad and Tobago and Mexico, and with a great influence of the African and Asian cultures – this pharmacological report is associated only with the traditional use of the plant by African slaves for the treatment of infectious diseases, mainly, intestinal disorders. At present, this use is merely restricted to a few mountainous areas and is not usually reported in local ethnobotanical studies. It is well known that different climatic, ground and growing conditions can modify qualitatively and quantitatively the chemical composition of the plant and therefore its pharmacological uses. In the specific case of tamarind, a difference in the chemical composition of the fruit pulp essential oil was found between the species that grows in Cuba and the one that grows in Egypt.

In a recent work conducted by our research group on leaves, we reported, for the first time, a total of 13 essential oils in which benzyl benzoate and limonene are the major compounds, followed by hexadecanol and pentadecenyl. It is widely accepted that essential oils are one of the plant's main secondary metabolites involved in antimicrobial and antiseptic activities, in which thyme oil is one of the most significant. Leaves also present good levels of protein, fat, fiber, and some vitamins such as thiamine, riboflavin, niacin, ascorbic acid and β -carotene. Flavonoid and other polyphenols are metabolites that have been also found in tamarind leaves; these compounds have a proven record as antimicrobial agents in many other plants.

Tamarindus Indica is indigenous to tropical Africa, particularly in Sudan, where it continues to grow wild; it is also cultivated in Cameroon, Nigeria and Tanzania. In Arabia, it is found growing wild in Oman, especially Dhofar, where it grows on the sea-facing slopes of mountains. It reached South Asia likely through human transportation and cultivation several thousand years prior to the Common Era. It is widely distributed throughout the tropical belt, from Africa to South Asia, Northern Australia, and throughout Oceania, Southeast Asia, Taiwan and China. In the 16th century, it was heavily introduced to Mexico, and to a lesser degree to South America, by Spanish and Portuguese colonists, to the degree that it became a staple ingredient in the region's cuisine. Today, South Asia and Mexico remain the largest consumers and producers of tamarind.

Taxonomical Classification:

Kingdom : Plantae
Phylum : Spermatophyte
Class : Angiosperm
Sub class : Dicotyledon
Family : Leguminosae
Subfamily : Caesalpinaceae
Genus : *Tamarindus*
Species : *Indica*

Microscopy:

The tamarind is a long-lived, medium-growth, bushy tree, which attains a maximum crown height of 12 to 18 metres (40 to 60 feet). The crown has an irregular, vase-shaped outline of dense foliage. The tree grows well in full sun in clay, loam, sandy, and acidic soil types, with a high drought and aerosol salt (wind-borne salt as found in coastal areas) resistance.

Leaves are evergreen, bright green in color, elliptical ovular, arrangement is alternate, of the pinnately compound type, with pinnate venation and less than 5 cm (2 inches) in length. The branches droop from a single, central trunk as the tree matures and is often pruned in human agriculture to optimize tree density and ease of fruit harvest. At night, the leaflets close up.

The tamarind does flower, though inconspicuously, with red and yellow elongated flowers. Flowers are 2.5 cm wide (one inch), five-petalled, borne in small racemes, and yellow with orange or red streaks. Buds are pink as the four sepals are pink and are lost when the flower blooms.

Tamarind is harvested by pulling the pod from its stalk. A mature tree may be capable of producing up to 175 kg (350 lb) of fruit per year. Veneer grafting, shield (T or inverted T) budding, and air layering may be used to propagate desirable selections. Such trees will usually fruit within three to four years if provided optimum growing conditions.

Macroscopy:

The fruit is an indehiscent legume, sometimes called a pod, 12 to 15 cm (3 to 6 inches) in length, with a hard, brown shell.^{[4][5][6]} The fruit has a fleshy, juicy, acidulous pulp. It is mature when the flesh is coloured brown or reddish-brown. The tamarinds of Asia have longer pods containing six to 12 seeds, whereas African and West Indian varieties have short pods containing one to six seeds. The seeds are somewhat flattened, and glossy brown.

The tamarind is best described as sweet and sour in taste, and is high in tartaric acid, sugar, B vitamins and, oddly for a fruit, calcium.

As a tropical species, it is frost sensitive. The pinnate leaves with opposite leaflets give a billowing effect in the wind. Tamarind timber consists of hard, dark red heartwood and softer, yellowish sapwood.

Description



Uses of various parts of *T. Indica*

Fruit pulp

Tamarind is valued mostly for its fruit, especially the pulp, which is used for a wide variety of domestic and industrial purposes. The acidic pulp is used as a favorite ingredient in culinary preparations, such as curries, chutneys, sauces, ice cream, and sherbet in countries where the tree grows naturally. In India, the pulp is also eaten raw and sweetened with sugar. Tamarind pulp is also used to make sweet meats mixed with sugar called Tamarind balls. Tamarind pulp is used as a raw material for the manufacture of several industrial products, such as Tamarind Juice Concentrate, Tamarind Pulp Powder, tartaric acid, pectin, tartarates, and alcohol.

Seed

Tamarind seed is a by-product of the commercial utilization of the fruit, the seed comprises the seed coat or testa (20-30%) and the kernel or endosperm (70-75%). However, it has several uses. It is commercially available as a food additive for improving the viscosity and texture of processed foods. The name “jellose” has been suggested for the seed polysaccharide as it describes both its jelly forming properties and the carbohydrate character. It has been recommended for use as a stabilizer in ice-cream, mayonnaise, and cheese and as an ingredient or agent in a number of pharmaceutical products, and the seed oil is said to be palatable and of culinary quality. The oil is used for making varnish to paint idols, and light lamps.

Flowers and leaves

The leaves, flowers, and immature pods of Tamarind are also edible. The leaves and flowers are used to make curries, salads, stews, and soups in many countries, especially in times of scarcity. These are used in some Thai food recipes because of their sourness and specific aroma. Children in Gambia mix the acid leaves with gum from fig trees to make a chewing gum. The leaves and flowers are also useful as a mordant in dyeing. A yellow dye derived from the leaves colors wool red and turns indigo dyed silk to green. Mature leaves are used as a bleaching agent in the preparation of young leaves of “buri” (*Corypha alata*) for hat making in the Philippines.

Wood

Tamarind wood has many uses, including making furniture, wheels, mallets, rice pounders, mortars, pestles, ploughs, well construction, tent pegs, canoes, side planks for boats, cart shafts and axles, and naves of wheels, toys, oil presses, sugar presses, printing blocks, tools and tool handles, turnery, and so on. In North America, Tamarind wood has been traded under the name of “Madeira mahogany” It is valued for making gunpowder. The ash is used to remove hair from animal hides. and can be mixed with fruit pulp for cleansing and brightening brass and copper vessels.

Seed testa and bark

The seed testa contains 23% tannin, in leather tanning tests, Tamarind tannin gives harsh and less, suitcases, and others. The seed husk has also been found to be an effective fish poison. Bark tannins are used in the preparation of ink and for fixing dyes. highly colored leather, which could be used for heavy so

Tamarind kernel powder

Tamarind Kernel Powder (TKP) produced from the seeds is another commercial product and is often reported in commercial digests. The TKP will become rancid and brown if stored inadequately and the storage ability and color will be better if it is defatted. In India, TKP is used as a source of carbohydrate as the adhesive or binding agent in paper and textile sizing, and weaving and making jute products, as well as textile printing.

Materials & Methods:

Plant Collection

Leaves of plant *Tamarindus indica* were collected from JNTU-H CAMPUS Hyderabad dist, in the month of April 2014. The Plant material was identified and authenticated by Dr. (Mr), Badraiah and Ramchandra Reddy Head-Department of Botany, Osmania University Campus, and Hyderabad. Authentication voucher No: 01711, S.No: 175. The leaves were dried under shade and were ground to a coarse consistency in a mixer-grinder and kept in an airtight container.

Preparation of Plant Extract

The powdered leaves of *Tamarindus indica* were successively extracted using solvents in order of increasing polarity, viz. acetone and ethanol. After extraction, each time the marc was dried and later extracted with next solvent. The two extracts were dried by distilling the solvents in rotary vacuum evaporator.

Phytochemical Screening: (Kokate, 2004)

Alkaloids: Mayer's test: To 2-3ml of extract, few drops of Mayer's reagent (1.36gm of Mercuric chloride and 5gm of Potassium iodide in 100ml distilled water) were added. Formation of cream color precipitated the presence of alkaloids.

Amino acids: Million's test: To 2ml of the test extract, about 2ml of Million's reagent (Mercury nitrate) were added. White precipitate indicates the presence of amino acids.

Carbohydrates: Molish test: To 2ml of test extract, at first, 2 drops of alcoholic alpha naphthol were added. Then through sides of the test tube few drops of concentrated Sulphuric acid were mixed with it. Purple to violet colour ring appeared at the junction indicate the presence of carbohydrates.

Flavonoids: Alkaline reagent test: To 2ml of test extract, few drops of sodium hydroxide solution were added. At first intense yellow colour formed, which was subsequently turned to colorless, on addition of few drops of dilute acid indicate the presence of flavonoids.

Glycosides: Borntrager's test: The test extract was boiled with 1ml of sulphuric acid in a test tube. While hot it was filtered and then cooled. Shaking of mixture was done with equal volume of chloroform. Two layers of solution were formed. The lower layer of chloroform was separated. Then that layer was shaken with half of its dilute ammonia. Production of rose pink to red colour indicates the presence of glycosides.

Saponins: Froth formation test: 2ml of test extract was shaken vigorously with water in a test tube. Formation of persistent foam indicates the presence of Saponins.⁽⁶⁾

Tannins: Gelatin test: To 2ml of test extract, 1% gelatin solution containing 10% Sodium chloride solution was added. Formation of precipitate suggested the presence of tannins.

Proteins: Warming test: 2ml of test extract was heated on a boiling water bath. Proteins get coagulated due to heating.

Steroids and Tri terpenoids: Salkowski test: The extract was treated with few drops of concentrated sulphuric acid. Red colour at lower layer indicates the presence of steroids; whereas formation of yellow colour at lower layer indicates the presence of triterpenoid.

Antibacterial activity using disc diffusion method:

Antimicrobial activity of the aqueous and organic extracts of the plant sample was evaluated by the paper disc diffusion method. For determination of antibacterial activity, bacterial cultures were adjusted to 0.5 McFarland turbidity standard and inoculated onto Nutrient agar (oxid) plates (diameter: 15cm). For the determination of antimycotic activity, all the fungal isolates and *Candida albicans* were first adjusted to the concentration of 10⁶ cfu/ml. Cultures of *Candida albicans* were suspended in sterile solution of 0.9% normal saline and the spores of the other filamentous fungi were suspended in Tanquay buffer and all the cultures were inoculated onto Sabouraud Dextrose Agar plates. Sterile filter paper discs (diameter 6mm for bacteria and 13mm for fungi) impregnated with 100µl of extract dilutions reconstituted in minimum amount of solvent at concentrations of 50 and 100mg/ml were

applied over each of the culture plates previously seeded with the 0.5 McFarland and 10⁶ cfu/ml cultures of bacteria and fungi respectively.

Bacterial cultures and those of *Candida albicans* were then incubated at 37°C for 18 h while the other fungal cultures were incubated at room temperature (30 – 32°C) for 48 h. Paper discs impregnated with 20 µl of a solution of 10 mg/ml of ciprofloxacin and cotrimoxazole (for bacteria) and nystatin and amphotericin B (for fungi) as standard antimicrobials were used for comparison. Antimicrobial activity was determined by measurement of zone of inhibition around each paper disc. For each extract three replicate trials were conducted.

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) of the extracts was estimated for each of the test organisms in triplicates. To 0.5 ml of varying concentrations of the extracts (20.0, 18.0, 15.0, 10.0, 8.0, 5.0, 1.0, 0.5, 0.05 and 0.005 mg/ml), 2 ml of nutrient broth was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard for (bacterial isolates) and 10⁶ cfu/ml (for fungal isolates) was introduced to the tubes. The procedure was repeated on the test organisms using the standard antibiotics (ciprofloxacin and cotrimoxazole for bacteria and nystatin and amphotericin B for fungal isolates). A tube containing nutrient broth only was seeded with the test organisms as described above to serve as control. Tubes containing bacterial cultures were then incubated at 37°C for 24 h while tubes containing fungal spore cultures were incubated for 48 h at room temperature (30 – 32°C). After incubation the tubes were then examined for microbial growth by observing for turbidity. To determine the MBC, for each set of test tubes in the MIC determination,⁽⁷⁾ a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar (for bacteria) and Sabouraud dextrose agar (for fungi) by streaking. Nutrient agar and Sabouraud agar only were streaked with the test organisms respectively to serve as control. Plates inoculated with bacteria were then incubated at 37°C for 24 hours while those inoculated with fungi were incubated at room temperature (30 – 32°C) for 48 h. After incubation the concentration at which no visible growth was seen was noted as the minimum bactericidal concentration.

Effect of Temperature and pH on antimicrobial activity of extracts

Five milliliters of 100 mg/ml of acetone extracts were constituted in test tubes and treated at 4, 30, 60 and 100°C in a water bath for 30 minutes and tested for antimicrobial activity. To determine the effect of pH, acetone extracts were treated at pH ranges of 2.5 to 10 using 1N HCl and 1N NaOH solutions respectively in series of test tubes for 30 minutes. After 30 minutes of treatment, each of the treated extracts were neutralized (pH 7) using 1N HCl and 1N NaOH as the case may be, and then tested for antimicrobial activity.

RESULTS & DISCUSSIONS:-

Results of Antimicrobial activities of extracts of *Tamarindus Indica*:

S.No	Organism	Water extract	Acetone extract	Ethanol extract	cotrimoxazole	Ciprofloxacin
1	<i>Escherichia coli</i>	6	10	8	18	32
2	<i>Staphylococcus aureus</i>	2	11	9	5	27
3	<i>Bacillus subtilis</i>	4	9	8	25	35
4	<i>Aspergillus Niger</i>	-	-	-	NA	NA
5	<i>Candida albicans</i>	-	-	-	NA	NA

Results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ethanolic extracts of Tamarindus Indica

S.No	ORGANISM	Organism MIC (mg/ml)	Leaf extracts MBC mg/ml)
1	<i>Escherichia coli</i>	15.5	18
2	<i>Staphylococcus aureus</i>	8	20
3	<i>Bacillus subtilis</i>	8	18
4	<i>Aspergillus niger</i>	-	-
5	<i>Candida albicans</i>	-	-

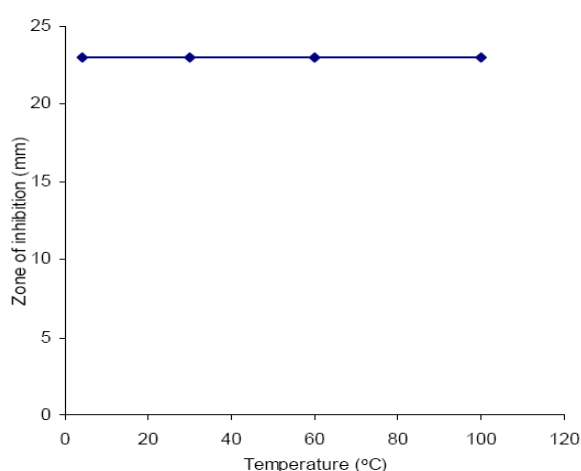


Fig 1. Effect of temperature on antimicrobial activity of *Tamarindus indica*

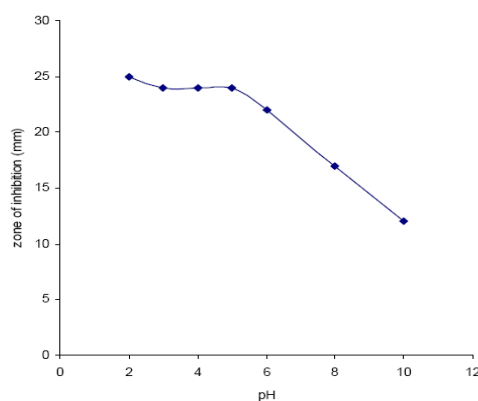


Fig 2. Effect of pH on antimicrobial activity of *Tamarindus indica*

Result of the effect of temperature and pH on the plant extracts showed that various temperature ranges of 4, 30, 60 and 100°C had no effect on the antimicrobial activity of the extracts (Fig 1), but the activity slightly increased at acidic pH (2 to 6). While at alkaline pH the activity of the plant extracts reduced (Fig 2). Results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are shown in Table 2. The result showed that *Staphylococcus aureus* had the highest MIC (18 mg/ml) and MBC (17.5 mg/ml), while the lowest MIC of 8 mg/ml was shown by *Bacillus subtilis*.

Plant Extract:

Following are the yields of the extracts in various solvents.

Solvent	% Yield	Colour	Physical status
Acetone	1.6	Light green	Solid
Water	2.4	Light green	Solid
Ethanol	11.8	Brownish green	Semi solid

PHYTOCHEMICAL SCREENING:

Preliminary phytochemical screening of *Tamarindus Indica* leaves extracts

Phytoconstituent	cold water extract	hot water extract	Ethanol extract
Alkaloids	-	-	+
Carbohydrates	++	++	+
Flavonoids	-	-	+
Tannins	+	+	++
Glycosides	-	-	+
Saponins	+	+	++
Sugars	++	+	+
Terpenes	-	-	-

(+)Present, (-) Absent For preliminary phytochemical screening of the extracts, we performed tests for alkaloids, carbohydrates, flavanoids, tannins, glycosides, Saponins, sugars and terpenes.

Summary and Conclusion:

In the present study, methods, techniques that address some current advantages in morphological, micro-chemical Characterization of plant. One of the major objectives is to identify potential and useful methods suitable to carry out anti platelet aggregation activity, anti-diabetic activity.

In first part of the present study, extraction of active constituents using solvents (hexane, chloroform and methanol) and preliminary phytochemical screening of various leaf extracts was presented. For preliminary phytochemical screening of the extracts, we performed tests for alkaloids, carbohydrates, flavanoids, tannins, glycosides, Saponins, proteins and steroids. While in case of hexane extract, carbohydrates, flavanoids, tannins, glycosides, steroids were detected, in case of chloroform extract, alkaloids, carbohydrates, flavanoids, tannins and steroids were found , alkaloids, flavanoids, tannins, glycosides, Saponins and steroids detected.

In conclusion, our findings show that chloroform leaf of of *tamarindus indica* extract reduction on blood glucose may be due to several lavanoids, glycosides present.⁽⁸⁾

Tamarind leaves are worldwide reported for their antioxidant and antimicrobial activity, but it hasn't been possible to establish a relationship with the chemical composition due to the scanty information availed. In this study, we detected eight components not previously reported, and confirmed the high fatty acid and polyphenol production in *Tamarindus indica* L leaves. In addition, high concentration of the most prominent pro-oxidant/antioxidant cations is described. All this information give light to the pretended intention to find chemical proof that support the pharmacological activities previously described for tamarind leaves.

Our study showed that there is great variation in fatty acids, elemental composition and total protein in *T. indeca*.

Moreover As, Pb, Cd were not detected by previous works and in recent investigation 32 fatty acids were isolated from same plant. The total protein analysis of *T. indica* medicinal plants from Jamshoro Distt. (Sindh) 15.6%. The great variation of fatty acids, elements and total protein is due to the environmental and ecological factors.

Present study revealed that *Tamarindus indica* possesses antidiabetic and hepatoprotective activity in alloxan induced diabetic rats. From the result, it is concluded that the juice of *Tamarindus Indica* linn leaves showed significant anthelmintic activity. When compared with the standard anthelmintic drug the drug may be further explored for its phytochemical profile to identify the active constituent responsible for anthelmintic activity.

REFERENCES:

1. Bibitha B, Jisha VK, Salitha CV, Mohan S, Valsa AK. Antibacterial activity of different plant extracts. Short Communication. *Indian J Microbiol* 2002; 42:361-363.
2. Maghrani, M.; Zeggwah, N.; Michel, J. and Eddouks, M. Antihypertensive effect of *Lepidium sativum* spontaneously hypertensive rats. *J Ethnopharm* 2005; 102 (1-2):193-197.
3. Predrag L, Hui S, Uri C, Hasswan A, Arieh B. The effects of aqueous extracts prepared from leaves of *Pistacia lentiscus* in experimental liver disease. *J Ethnopharm* 2005; 100 (1-2): 198-204.
4. Aida P, Rosa V, Blamea F, Tomas A, Salvador C. Paraguayan plants used in traditional medicine. Short communication. *J. Ethnopharm* 2001; 16: 93-98.
5. Lutterodt GD, Ismail A, Basheer RH, Baharudin HM. Antimicrobial effects of *Psidium guajava* extracts as one mechanism of its antidiarrhoeal action. *Malaysian J Med Sci* 1999; 6 (2):17-20.
6. Marjorie MC. Plant products as antimicrobial agents. *Clin Microbiol Rev* 1999., 12(4): 564-582.
7. Srinivasan D, Perumalsamy LP, Nathan S, Sures T. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J Ethnopharm* 2001; 94: 217-222.
8. Molan, PC. The antibacterial activity of honey 1. The nature of antibacterial activity. *Bee World* 199.
9. Lai PK, Roy J. Antimicrobial and chemopreventive properties of herbs and spices. *Curr. Med. Chem.* 2004; 11:1451-1460.
10. Ferrara L. Antioxidant activity of *Tamarindus indica*. *Ingredient alimentary*. 2005; 4:13-5.
11. Hydrotropic technique: a promising method to enhance aqueous solubility of nimesulide and to reduce difficulties in bioavailability, *asian journal of pharmaceuticals*, 12 (4) | s1-s7, 2018.
12. Design, formulation, and evaluation of sustained release tablets for antihyperlipidemic agent, *asian journal of pharmaceuticals*, 12 (4) | 312-319, 2018.
13. Effect of analgesic activity in crude extract and isolated compounds of *tecomaria capensis*, *international journal of green pharmacy*, 12 (4) | 322-328, 2018.
14. In vitro and in vivo anti-inflammatory potential of isolated compounds from ethyl acetate extracts of *tecomaria capensis*, *international journal of green pharmacy*, 12 (4) | s1-s7, 2018.
15. Enhancement of dissolution rate and formulation development of pioglitazone-abc class-a drug, *journal of pharmacy research*, 4(11), 3862-3863, 2011.

- 16 Formulation and characterization of colon specific drug delivery system of a matrix tablet, world journal of pharmaceutical and life sciences, vol. 2, issue 2, 330-348, 2016.
- 17 Lipid polymer based nano particles for the therapy of ulcerative colitis to improve the therapeutic efficiency, journal of emerging technologies and innovative research, volume 5, issue 8,1-12, 2018
- 18 Lipid polymer based nano particles for the treatment of ulcerative colitis-review, international journal of research and analytical review, volume 6, issue 2, 549-568, 2019
- 19 Lipid-polymer based nanoparticles as a new generation therapeutic delivery platform for ulcerative colitis in vitro/in vivo evaluation, international journal of innovative technology and exploring engineering & sciences, volume 21, issue 1, 42-46,2019

