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Individual and combined effects of extracts of Leaves and Bark of *Tamarindus indica* on invitro membrane stabilizing and antioxidant activities

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Abstract

The use of natural products with therapeutic properties is as ancient as human civilisation and, for a long time, mineral, plant and animal products were the main sources of drugs. Herbal medicines are now in great demand in the developing world for primary health care not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. In present study have investigated Individual and Combined effects of extracts of leaves and bark of *Tamarindus indica* on invitro membrane stabilizing and antioxidant activities.The method for membrane stabilizing activity was done by hypotonic solution induced haemolysis and method for antioxidant activity was done by DPPH method. From the result, we foundout that total methanol extract of leaves showed maximum membrane stabilizing activity 48.23±1.07 % as well as antioxidant activity 91.39±1.17 %. Upon combination of of methanol extract of bark & leaves showed significant membrane stabilizing and antioxidant activity.

Keywords: *Tamarindus indica,* Erythrocyte, Membrane stabilzation, antioxidant, anti-inflammatory, Ascorbic acid, Aspirin, DPPH.

INTRODUCTION

Since ancient times herbal medicines have been used for the treatment of various diseases. Medicinal plant have important contribution and play a key role in healthcare system specially in a recent decades having great development and advancement in medicine[1].

According to WHO, about quarter of the world population rely on herbal resources in their primary helathcare[2,3]. Natural products are being used in traditional medicine and are of great importance. Many traditional medicine system all over the world have been used natural product and have been practiced for hundreds or even thousands of years, and have developed as a orderly-regulated systems of medicine[4,5]. All over the world medicinal plants are an important source of indigenous medicinal systems[6]. Herbal plants provides rich resources for natural drug research and development. Herbal medicines used as a traditional herbal medicine because of traditional practice, substantial historical use, cultural reasons and maintained their popularity. Although Modern medicine also exist with such traditional medicine[7]. Natural products have great importance all over the world in the treatment and prevention of human diseases. Natural prodcts medicines obtained from various resources such as terrestrial micro-organisms, marine organisms and terrestrial vertebrates and in vertebrates[8]. More than 70% of the total population of India uses herbal drugs for the treatment of diseases. Herbal medicine use different phytoconstituents of medicinal plants for the prevention and treatment of diseases. Herbal medicine is cheap because of its easy availability. Many plant derived metabolite like opium, quinine, digitalis etc. use in pharmaceutical drugs as a clinical medicines[9].

Tamarindus indica Linn, commonly called as Imli in Hindi, is known as Chincha or Amlika in Ayurveda and the member of Caesalpinaceae subfamily of Fabaceae family. It is distributed throughout India, particularly in the south, often cultivated. The tree averages 20-25 m in height and 1 m in diameter, slow growing, but long lived, with an average life span of 80-200 years. Virtually every part of *Tamarindus indica* L. (wood, root, leaves, bark and fruits) has either nutritional or medicinal value, with a number of industrial and commercial applications[10, 11].

Tamarind is useful in gastric disorders, bilious vomiting, scurvy, datura poisoning, alcoholic intoxication, scabies, pharyngitis, otalgia, stomatitis, constipation, haemorrhoids and eye diseases[12]. Tamarind pulp is also said to aid in the cure of malarial fever[13]. The fruits are reported to have hypolipidemic, anti-inflammatory, antifungal and antibacterial properties[14]. It is a large tree, attaining 60-80 feet in height and bearing a very large, widely spreading head of foliage, trunk with a dark rough bark, youngest twigs smooth or slightly pubescent, flowers are in bunches, yellow in color and boat shaped[15]; seeds are reddish brown, thick; the flattened sides of the seeds are marked by a centrally placed dull area[16]; fruit pulp occurs as a reddish-brown, moist, sticky mass, in which yellowish-brown fibers are readily seen; odour is pleasant, taste is sweetish and acidic[15,17]; bark of the trunk is scaly[18]; leaves are paripinnate up to 15 cm long[19]. Various pharmacological activity has been reported anti snake venom activity[20], hepatoprotective effect[21], analgesic activity[22], hypolipidemic activity[23], anti-helminthic activity[24], anti-inflammatory and analgesic activity[25], hypoglycemic properties[26], anti-microbial, antifungal and anti-melioidosis activity[27].

Oxidation reactions are very important for plants and animals but these reactions can also be damaging; hence multple types of complex antioxidant system maintain by plant and animals such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidase. Oxidative stress may cause damage and kill of cells because of reduced level of antioxidants or inhibition of antioxidants enzymes. Oxidative stress is reason for many types of diseases. Antioxidants are used in pharmacology is intensively studied, particularly as treatment for stroke and neurodegenerative diseases. So oxidative stress is very important part of human diseases[28]. As antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electron from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radicals intermediates, and inhibit other oxidation agents such as thiols, ascorbic acid or polyphenols[29]. In general, the reactive oxygen species circulating in the body tend to react with the electron of other molecules in the body and these also effect various enzyme systems and cause damage which may further contribute to conditions such as cancer, ischemia, aging, adult respiratory distress syndromes, rheumatoid arthritis etc[30]. The most common reactive oxygen species include superoxide anion (O_2), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO) and reactive hydroxyl radicals (OH). The nitrogen derived free radicals are nitric oxide (NO), peroxy nitrite anion (ONOO), Nitrogen dioxide (NO_2) and Dinitrogen trioxide (N_2O_3)[31].

Inflammation is due to response of alergens and injury to the tissues. It is defense response of our body where as disorders including allergies, cardiovascular dysfunctions, metabolic syndrome, cancer, and autoimmune diseases is the main cause of uncontrolled inflammation which imposing huge economic burden on individals and consequently on the society[30].

Inflammation is controlled and suppressed by various kinds of anti-inflammatory agents and immunosuppressant which are associated with various adverse efffects are the practical examples of these medications. While in practice our goal is to apply minimum effective dose by the highest efficacy with the least adverse effects. Thus, we need to apply natural anti-inflammatory factors within medication therapy to achieve increased pharmacological response and the lowest degree of unwanted side effects[30,32]. From the above aim, the selection of plant for evaluation was based on its traditional uses and evaluated for the its membrane stabilizing and antioxidant activity. From the above aim, the selection of plant for evaluation was based on its traditional uses and evaluated for the its membrane stabilizing and antioxidant activity.

MATERIALS ANS METHODS

Collection and Identification of leaves & bark of *Tamarindus indica*:

Leaves and Bark of *Tamarindus indica* were collected from locality of Muzaffarnagar,U.P (India). Plant material was authenticated by Dr. Vidit Tyagi, Botanist, Dept. of Botany, Dolphin PG Institute of Biomedical and Natural Sciences, Dehradun, Uttarkhand.

Preparation of leaves extract and fractions

The collected plant material was washed with water to remove other undesirable material and dried under shade.The air-dried leaves (1000 gm) of *Tamarindus indica* were crushed. The crushed leaves extracted with methanol by cold percolation method using percolator. The extract was evaporated till dryness to obtain a residue. From total methanol extract, different fractions were prepared by successive fractionation (separation technique) using increasing polarity of solvents i.e. Petroleum Ether, Chloroform, Ethyl Acetate & n-Butanol.

Preparation of bark extract of *Tamarindus indica*

The collected plant material was washed with water to remove other undesirable material and dried under shade. The air-dried bark (370 gm) of *Tamaridus indica* were crushed. The crushed bark extracted with methanol by hot percolation method using soxhlet apparatus. The extract was evaporated till dryness and concentrated under reduced pressure to obtain residue.

Phytochemical analysis of extracts of leaves and bark

Extracts of leaves and bark of *Tamaridus indica* were subjected to evaluate the presence of different phytoconstituents such as alkaloids, carbohydrate, steroids, proteins-amino acids, saponin and phenolic compounds.

Invitro membrane stabilizing and antioxidant activities of extracts

Invitro membrane stabilizing activity of leaves and bark extracts[33-36]

Erythrocytic suspension

Whole blood was collected from goat from slaughter house and NIH-ACD (National Institute of Health-Acid Citrate Dextrose) solution was added to it to prevent clotting. The blood was centrifuged three times with 0.9% saline. The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (pH 7.4). Which contained in 100 ml of distilled water: $NaH_2PO_4.2H_2O$, 0.26 g; Na_2HPO_4, 1.15 g; NaCl, 9 g (10 mM sodium phosphate buffer). The isotonic buffer solution was composed of 154 mM NaCl in 10 mM sodium phosphate buffer (pH 7.4).

Hypotonic solution-induced haemolysis

Stock erythrocyte suspension (30 µl) was mixed with 5 ml of the hypotonic solution containing the *Tamaridus indica* extracts and fractions at concentrations of 1000, 1500 and 2000 µg/ml. combined extract of both leaves and bark extracts were prepared at a ratio of 1:1 (w/w), while the control sample was mixed with drug free solution. The mixtures were incubated for 10 min at room temperature, and centrifuged at 3000 g for 10 min. All the experiments were performed in triplicates and the absorbance (O.D.) of the supernatant was measured at 560 nm. Aspirin was used as a reference standard.

Calculation

The percentage inhibition or acceleration of hemolysis in test was calculated according to the equation:

% acceleration or inhibition of hemolysis = 100 x $\left[\frac{OD1-OD2}{OD1}\right]$

Where, OD^1 = Optical density of hypotonic saline solution + blood (control) and OD^2= Optical density of test sample in hypotonic saline solution + blood

Antioxidant activity of leaves and bark extracts[37, 38]

Preparation of DPPH:

DPPH is a highly oxidisable compound. It oxidized in light, so DPPH is prepared in dark. Weigh accurately 20 mg DPPH and dissolved in 100 ml methanol.

Preparation of standard Ascorbic acid solution & different concentration of *Tamaridus indica* extracts:

Ascorbic acid is an strong antioxidizing agent. It is taken as standard. Standard solution of ascorbic acid as well as extracts is prepared. viz. 50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml. Combined extract of both leaves and bark extracts were prepared at a ratio of 1:1 (w/w).

Preparation of test sample & standard sample:

3 ml of different concentration of test sample *Tamaridus indica.* Extracts and standard (ascorbic acid) were mixed separately with 1 ml of DPPH solution in dark. The prepared solution of ascorbic acid and test sample was incubated for 1/2 half an hour. When procedure is done than absorbance is taken with the help of U.V. Spectrophotometer at 517 nm.

We calculate the % activity of individual concentration of individual extract from the following formula:-

$$\% \text{ Activity} = \frac{\text{Abs. of control} - \text{Abs. of individual concentration}}{\text{Abs. of control}} \times 100$$

Abs. = Absorbance

RESULTS AND DISCUSSION

The air dried bark (370gm) of *Tamarindus indica* extracted with methanol by hot percolation method using soxhlet assembly. The extract was evaporated till dryness to obtain methanol extract 9.89gm.

The collected leaves of *Tamarindus indica* was dried under shade. The air-dried leaves (1000 gm.) of *Tamarindus indica* were crushed. The crushed leaves extracted with Methanol by cold percolation method using percolator. The extract was evaporated till dryness to obtain a residue of 69 gm. From total methanol extract, different fractions were prepared by successive fractionation (separation technique) using increasing polarity of solvents & yield were, Petroleum Ether (15.7 gm), Chloroform (6.72 gm), Ethyl acetate (3.84 gm) & n-Butanol (16.5 gm) yield.

The methanol extract of bark and methanol extract & its fractions of leaves of *Tamarindus indica* undergo various qualitative phytochemical tests. we found out that total methanol extract of leaves was the richest extract for phytoconstituents. It contains maximum tested phytoconstituents viz. Alkaloids, carbohydrates, phenolic compounds, except saponin and protein and amino acids. Petroleum ether and chloroform extracts showed the presence of sterols and carbohydrates repectively. Ethyl acetate and Butanol extracts showed presence of alkaloids, carbohydrates and sterols except phenolic compounds, saponin, proteins and amino acids.

Membrane Stabilizing activity:

Membrane stabilizing activity of the bark and leaves extract & its fractions were compared with activity of standard drug Aspirin. It was observed that the concentration of 2000 µg/ml of Total methanol extract of leaves showed maximum membrane stabilization activity 48.23±1.07 percent. The butanol fraction showed the 47.5±1.07 percent activity. While Combined extract of methanol bark and methanol extract of leaves showed 43.07±0.95 percent activity (Table 1).

Release of lysosomal constituents are cause of Inflammation which cause damage of cell. Erythrocyte membrane resemblance the lysosomal membrane. By stabilization of lysosomal membrane inhibits the release of lysosomal constituents. So by stabilize the erythrocyte membrane with extracts may also stabilize lysosomal membrane[39,40]. Stabilization of erythrocyte cell membrane by hypotonic solution induced erythrocyte membrane lysis can be taken as an invitro measure of anti-inflammatory activity of the drugs or plant extracts.

Antioxidant activity

Total methanol extract of leaves of *Tamarindus indica* showed maximum antioxidant activity in comparision to all extracts. The concentration of 500 µg/ml of total methanol extract of leaves showed 91.39±1.17 percent antioxidant activity (Table 4). Combined extract of total methanol leaves and methanol bark showed 90.24±1.48 percent antioxidant activity at a concentration of 500 µg/ml (Table 2).

The change in colour of DPPH solution is due to radical species and antioxidants scavenges by DDPH molecule which depends on the concentration and potency of the antioxidants. Low absorbance of the reeaction mixture indicates significantly the increase in antioxidant activity[41].

In the present study the combined total methanol extract of leaves and methanol extract of bark and also combined ethyl acetate extract of leaves and methanol extract of bark were taken at a ratio 1:1 (w/w) to check the effect of membrane stabilizing and antioxidant effect. The main aim of the above is to check the synergestic effect of active extracts in combined form and from the study we found out that the combined extract of methanol of leaves & methanol bark extract showed significant maximum antioxidant activity 88.66±1.06 among all the extracts.

Table 1: Effect of different extract and standard drug on membrane stabilizing activity:

Concentration of extracts (µg/ml)	% Membrane stabilizing activity of extracts & standard drug								
	Tamarindus indica leaves extracts					*Tamarindus indica* bark extract	Combined extract of *Tamarindus indica* Leaves & Bark	Standard Drug	
	Petroleum ether	Chloroform	Ethyl acetate	Butanol	Total Methanol extract	Methanol Bark	Methanol Leaves + Methanol Bark	Acetyl Salicylic acid	Concentration of Acetyl Salicylic acid (µg/ml)
1000	16.43±0.84	19.67±0.5	21.56±1.27	28.13±1.57	19.62±0.72	6.64±0.76	18.64±1.0	49.14±0.77	**100**
1500	21.26±1.01	28.33±1.06	39.77±1.76	36.70±1.13	39.70±0.74	14.23±0.75	31.32±0.67	55.66±0.75	**150**
2000	25.23±1.3	37.73±0.95	43.12±1.14	47.5±1.07	48.23±1.07	21.47±0.33	43.07±0.95	58.13±0.71	**200**

Results are expressed as mean values ± standard error (n = 3)

Table 2: Effect of different extract and standard drug on antioxidant activity:

Concentration of extracts (µg/ml)	% Antioxidant activity of extracts & standard drug								
	Tamarindus indica Leaves extracts					*Tamarindus indica* Bark extract	Combined extract of *Tamarindus indica* Leaves & Bark		Standard Drug
	Petroleum ether	Chloroform	Ethyl acetate	Butanol	Total Methanol extract	Methanol Bark	Methanol Leaves + Methanol Bark	Ethyl acetate Leaves + Methanol Bark	Ascorbic Acid
50	21.93±0.96	23.24±1.67	29.63±0.48	44.97±1.17	28.58±1.14	20.44±0.62	10.58±0.51	38.84±0.80	96.50±0.19
100	29.88±0.89	30.31±0.86	43.01±1.35	50.94±1.4	39.43±0.77	24.54±1.19	44.58±1.07	69.42±0.57	96.45±0.11
200	33.37±1.48	40.12±1.49	55.47±1.62	78.69±0.94	61.70±1.90	37.30±1.32	74.47±1.29	73.65±0.63	96.67±0.17
300	52.42±0.89	52.93±1.39	72.37±1.10	83.41±1.6	77.36±1.07	54.66±1.05	80.69±1.26	81.72±0.85	96.25±0.17
400	54.88±0.55	60.56±1.25	79.13±2.07	86.92±1.06	87.56±1.17	58.53±1.22	88.73±0.78	85.34±1.83	96.25±0.17
500	59.01±1.4	67.61±0.70	88.71±1.10	89.41±0.48	91.39±1.17	69.76±0.58	90.24±1.48	88.66±1.06	96.49±0.16

Results are expressed as mean values ± standard error (n = 3)

CONCLUSION

From the above studies it could be concluded that total methanol extract of leaves showed maximum membrane stabilizing activity as well as antioxidant activity. Upon combination of of methanol extract of bark & leaves showed significant membrane stabilizing and antioxidant activity. So further study is needed for the isolation of active principle.

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