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Preliminary sceening of Anti inflammatory and Antioxidant activity of *Morinda umbellata*

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Abstract

The presesent work was undertaken to evaluate the preliminary phytochemical, antioxidant and anti-inflammatory activity of *Morinda umbellata*. Bovine serum albumin denaturation inhibition assay was used for the evaluation of in-vitro anti-inflammatory activity and DPPH Free radical scavenging and super oxide scavenging assays were used to assess the antioxidant activity of ethanol and aqueous extracts of the whole plant of *Morinda umbellata*. In anti-denaturation study it was observed that aqueous extract showed greater percentage of inhibition of bovine serum albumin denaturation i.e. 44.8% whereas ethanol extract showed 35.4% at the 400µg/ml concentration respectively. In case of antioxidant screening also, aqueous extract showed better antioxidant power compared to ethanol extract dose dependently. Among the two extracts evaluated for anti-inflammatory and antioxidant activities aqueous extract of *Morinda umbellata* was found to possess significantly good anti-inflammatory and antioxidant activities and this can be attributed to the presence of alkaloids, saponins, glycosides, flavonoids and phenolic compounds.

Key-Words: *Morinda umbellata*, anti-inflammatory, anti-oxidant, DPPH

Introduction

Plant dugs have been a major source for treatment of diseases for a long time. They have been used in the traditional medicine on the basis of experiences and practice. With the advent of modern systems of medicine need has been felt to investigate the active constituents present in these plants. Various molecules have been isolated, characterized and tested for their related pharmacological activities.

When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form of stress. The response to the stress of tissue damage is called as inflammation. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. Whether loss of function occurs depends on the site and extent of injury. Since inflammation is one of the body's nonspecific internal systems of defence, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion[1].

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Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. For example, enzymes lose their activity, because the substrates can no longer bind to the activesite[2].

Oxidative stress is induced by wide range of environmental factors including UV stress, pathogen invasion (hypersensitive reaction), herbicide action and oxygen shortage. Oxygen deprivation stress in plant cells is distinguished by three physiologically different states: transient hypoxia, anoxia and re oxygenation. Generation of reactive oxygen species (ROS) is the characteristic feature for hypoxia and essentially for re oxygenation. Of the ROS, hydrogen peroxide (H_2O_2) and superoxide (O^{2-}) are both produced in number of cell reactions, including iron catalysed Fenton-reaction and by various enzymes such as lipoxygenases, peroxidases, NADPH oxidase and xanthine oxidase. The main cellular components susceptible to damage by free radicals are lipids (peroxidation of unsaturated fatty acids), proteins (denaturation), carbohydrates and nucleic acids[3].

Both inflammation and free radical damage are inter-related aspects that influence each other. As said above proteins are susceptible to undergo denaturation by formation of free radicals and the mechanism of inflammation injury is attributed, in part, to release of reactive oxygen species from activated neutrophil and macrophages. This over-production leads to tissue injury by damaging the macromolecule and lipid peroxidation of membranes. In addition, ROS propagate inflammation by stimulating the release of the cytokines such as interleukin- I tumor necrosis factor-α, and interferon-γ, which stimulate recruitment of additional neutrophill and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation[4,5]. Thus our study aims to find a natural remedy that will be useful to treat both inflammation and free radical damage.

Material and Methods

Collection and Authentication

Morinda umbellata is a climber of Rubiaceae family. In Malayalam the plant is known as Ney-valli. It is distributed in high ranges of Western Ghats. The tribal communities use this plant for diabetes, high blood pressure, muscle aches and pain.

Plant material

Whole plants were collected from Wynad hills, Wynad district, Kerala, India. The taxonomical identification of the plant was done by Dr. N. Sasidharan, Scientist-F, Programme co-ordinator, FE & BC division, Kerala Forest Research Institute, Peechi, and Trissur. The voucher specimen was preserved in Academy of Pharmaceutical sciences, Pariyaram Medical College, Kannur, and Kerala.

Preparation of plant extract

The collected whole plant was dried at room temperature, pulverized by a mechanical grinder, sieved through 40 meshes. About 200g of powdered materials were extracted with ethanol and water. The extracts are then concentrated using vacuum evaporator and dried under reduced pressure.

Chemicals

Bovine serum albumin (BSA) 5%, Phosphate buffer saline (pH 6.3), EDTA, Nitro blue tetrazolium (NBT), Riboflavin, DPPH (2,2-diphenyl-1-picrylhydrazyl) and Indomethacin were purchased from Merck Pharmaceuticals.

Phytochemical screening

The concentrated extracts were used for preliminary screening of various phyto constituents vis, carbohydrates, amino acid, alkaloids, tannins, glycosides and flavonoids were detected by usual methods prescribed in standard tests[6, 7].

In vitro Bovine serum albumin denaturation inhibition assay[8]

The reaction mixture 3ml contained, 50µl of the test solution (100, 200, 400µg/ml was prepared in methanol). 450µl of 5% w/v BSA was added to all the above test tubes. For control tests, 50 µl of distilled water instead of test solution. The test tubes were incubated at 37ºC for 20 minute and then heated at 57ºC for 3 minutes. After cooling the test tubes, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. The absorbance of these solutions was determined by using spectrophotometer at a wavelength of 660nm.

% Protein Denaturation Inhibition = [(Abs of control – Abs of treated)/Abs of control] x100

DPPH Free Radical Scavenging Assay[9, 10, 11]

1ml of the test compound in various concentrations (50, 100,150, 200 & 250 µg/ml) was added to 1ml of 0.1mm solution of DPPH in methanol. After 30 minutes, absorbance was measured at 517nm, using a spectrophotometer (SHIMADZU, UV 1800). A 0.1 mM solution of DPPH in methanol was used as blank, whereas ascorbic acid was used as a reference standard. All tests were performed in triplicate. Percent inhibition was calculated using equation,

Percent inhibition = [(Control × Test)/Control] ×100

Superoxide Scavenging Assay[9, 10, 11]

1ml of the test compound in various concentrations (50, 100, 150, 200 & 250 µg/ml) was added to 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.6ml phosphate buffer. The control tubes were also set up where DMSO was added instead of the plant extracts. All the tubes were vortexed and the initial optical density was measured at 560 nm in a spectrophotometer. The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560 nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity. All tests were performed in triplicate. Percent inhibition was calculated using equation,

Percent inhibition = [(Control×Test) /Control] ×100

Results and Discussion

Phytochemical studies

From the phytochemical study, it has evaluated the presence of alkaloids, flavonoids, glycosides, phenols, and saponins. (Table 1)

In Vitro anti-inflammatory activity (Bovine serum albumin denaturation inhibition assay)

Both ethanol and aqueous extracts of *Morinda umbellata* inhibited the denaturation of Bovine serum

albumin (BSA). The degree of inhibition of BSA denaturation increased with the increase in the concentration of both the extracts, that the anti-denaturation of the drug will be more at higher concentration. As shown in Table 2 and 3 among the two extracts under the study aqueous extract (from 26.3±0.11% to 44.8±0.16%) has shown better inhibition of BSA denaturation at any concentration compared to ethanol extract (from 20.8±0.18% to35.4±0.11%). The standard drug Indomethacin showed 88.9±0.46% inhibition denaturation at 100 µg/ml concentration.

The method of anti-denaturation of BSA was chosen to evaluate anti-inflammatory property of *Morinda umbellata*. In anti-denaturation assay the denaturation of BSA is induced by heat treatment. The denatured BSA expresses antigens associated to Type III hyper-sensitive reaction which are related to diseases such as serum etc[12]. Heat denatured proteins are as effective as native proteins in provoking delayed hypersensitivity[13]. Moreover it was already proved that conventional NSAID's like phenylbutazone and indomethazine does not act only by the inhibition of endogenous prostaglandins production by blocking COX enzyme but also by prevention of denaturation of proteins[14]. Thus anti denaturation assay is the convenient method to check the anti-inflammatory activity.

In our results both the extracts (Table 2 and 3) has shown considerable anti-inflammatory activity and aqueous extract was found to be more potent than ethanol extract. The secondary metabolites like phenolic compounds and tannins which were found in preliminary phytochemical screening might be responsible for this activity.

Antioxidant activity

Several concentrations ranging from 50-250µg/ml of the ethanol and aqueous extracts of *Morinda umbellata* were compared for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the extracts in a concentration dependent manner up to the given concentration in all the models. The absorbance of the two extracts increased with the rise in their concentrations since the absorbance is directly proportional to the antioxidant property. As shown in table 4 and 5 among the two extracts under the study the aqueous extract (from 20.16±1.22% to 48.87±4.23% for DPPH free radical scavenging assay and from 22.18±2.10% to 51.16±4.13% for super oxide scavenging assay) indicates better antioxidant power compared to ethanol extract (from 19.89±1.44 % to 40.61± 4.03% for DPPH free radical scavenging assay and from 20.41±1.20% to 39.06±3.54% for super oxide scavenging assay) (Table 6 and 7). The standard drug ascorbic acid showed 72.78±3.97% antioxidant power at 50µg/ml concentration.

Free radicals are chemical entities that can exist separately with one or more unpaired electrons[15]. The propagation of free radicals brings about a myriad of reactions and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by these free radicals. The oxidative stress exerted due to these free radicals has been implicated in the pathology of various diseases like diabetes, inflammations, cardiovascular complications, cancer and ageing[16]. Antioxidants offer resistance against the oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation and by many other mechanisms and thus prevent disease[17].

In our study, both the extracts has shown considerable scavenging of free radicals in all the *in vitro* models studied. DPPH is a stable free radical. The *in vitro* study carried out on this radical is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH[18]. This radical reacts with suitable reducing agents, the electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up[19]. From the present results, it may be concluded that *Morinda umbellata* reduce the radical to the corresponding hydrazine when they react with the hydrogen donors in the antioxidant principles. (Table 4 and 5)

Superoxide dismutase catalysis the dismutation of highly reactive superoxide anion to oxygen and hydrogen peroxide [20]. Superoxide anion is the first reduction product of oxygen which is measured in terms of inhibition of generation of 0_2[21]. (Table 6 and7)

Preliminary phytochemical screening revealed the presence of alkaloids, phenolic compounds, tannins, glycosides and flavonoids in *Morinda umbellata*. Phenolic, flavonoids and tannins have been proved to be responsible for the antioxidant activity of various medicinal plants reported earlier[22,23,24]. Hence, these may be responsible for the observed activity. (Table 1)

Conclusion

Our investigation clearly demonstrates that ethanol and aqueous extracts of *Morinda umbellata* possess significant anti-inflammatory and antioxidant activity. Among them aqueous extract was found to be more potent than the ethanol extract. Further studies are recommended to isolate the active principle responsible for these activities.

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Table 1: Preliminary phytochemical screening of the aqueous and ethanol extracts of *Morinda umbellata*

Chemical constituents	Test	Aqueous	Ethanol
Alkaloid	Dragendroff's reagent	-	-
	Mayer's reagent	+	+
	Wagner's reagent	+	+
	Hager's reagent	+	+
Amino acid	Millon's test	-	-
Proteins	Lowry's method	-	-
Phenols	Folin test	+	+
Fats	Filter paper test	-	-
Carbohydrates	Molish test	+	-
	Barfoed's test	-	-
Starch	Iodine test	-	-
Saponins	Form test	+	+
Sugar	Anthrone Reagent Test	-	-
Steroids	Liebermann- Burchard test	+	+
Flavanoids	Ethyl acetate test	+	+
Cardiac glycosides	Ferric chloride test	+	+
Quinone	Sodium hydroxide test	+	-
Tannins	Feric Chloride Test	+	+

Table 2: Effect of aqueous extract of *Morinda umbellata* on in-vitro Bovine serum albumin denaturation inhibition

S/No.	Group	Concentration (µg/ml)	% Inhibition of denaturation
1.	Control	-	-
2.	Indomethacin	100 µg/ml	88.9±0.46
3.	Aqueous	100 µg/ml	26.3±0.11
4.	Aqueous	200 µg/ml	32.6±0.24
5.	Aqueous	400 µg/ml	44.8±0.16

Values are Mean± SEM ,n= 3

Table 3: Effect of ethanol extract of *Morinda umbellata* on in-vitro Bovine serum albumin denaturation inhibition

S/No.	Group	Concentration (µg/ml)	% Inhibition of denaturation
1.	Control	-	-
2.	Indomethacin	100 µg/ml	88.9±0.46
3.	Ethanol	100 µg/ml	20.8±0.18
4.	Ethanol	200 µg/ml	26.3±0.22
5.	Ethanol	400 µg/ml	35.4±0.11

Values are Mean± SEM, n=3

Table 4: Effect of aqueous extract of *Morinda umbellata* on in-vitro antioxidant activity by DPPH radicle scavenging assay

Concentration (µg/ml) of Chloroform extract	% Scavenging	Concentration (µg/ml) of Ascorbic acid	% Scavenging
50	20.16±1.22	10	35.63±2.32
100	28.91±2.13	20	48.69±2.43
150	36.01±3.12	30	53.67±2.52
200	42.14±3.20	40	61.91±3.57
250	48.87±4.23	50	72.78±3.97

Values are Mean ± SEM, n=3

Table 5: Effect of ethanol extract of *Morinda umbellata* on in-vitro antioxidant activity by DPPH radicle scavenging assay

Concentration (µg/ml) of aqueous extract	% Scavenging	Concentration (µg/ml) of Ascorbic acid	% Scavenging
50	19.89±1.44	10	35.63±2.32
100	24.63±2.02	20	48.69±2.43
150	29.97±3.04	30	53.67±2.52
200	34.26±3.53	40	61.91±3.57
250	40.61±4.03	50	72.78±3.97

Values are Mean ± SEM, n=3

Table 6: Effect of aqueous extract of *Morinda umbellata* on in-vitro antioxidant activity by superoxide scavenging assay

Concentration (µg/ml) of Chloroform extract	% Scavenging	Concentration (µg/ml) of Ascorbic acid	% Scavenging
50	22.18±2.10	10	35.63±2.32
100	30.98±2.22	20	48.69±2.43
150	38.14±3.32	30	53.67±2.52
200	44.22±4.02	40	61.91±3.57
250	51.16±4.13	50	72.78±3.97

Values are Mean ± SEM, n=3

Table 7: Effect of ethanol extract of *Morinda umbellata* on in-vitro antioxidant activity by superoxide scavenging assay

Concentration (µg/ml) of Aqueous extract	% Scavenging	Concentration (µg/ml) of Ascorbic acid	% Scavenging
50	20.41±1.20	10	35.63±2.32
100	25.32±1.19	20	48.69±2.43
150	29.73±2.64	30	53.67±2.52
200	33.12±3.11	40	61.91±3.57
250	39.06±3.54	50	72.78±3.97

Values are Mean ± SEM, n =3

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