Assessment of Herbal Ointment for Antioxident Activity in Wistar Rats

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Abstract:

Medicinal plants play an important role in treating various diseases. Cashew is an evergreen perennial plant belonging to the family Anacardiaceae. The aim of present study to formulate, evaluate and to check the antioxidant activity of newly prepared herbal ointment formulation. These Formulations were evaluated for the following parameters: pH, Spread ability, grittiness, skin irritation study, stability. The prepared herbal ointment formulation showed the antioxidant activity during evaluation of radical scavenging activity. It was expressed as the percentage of reduction of initial radical absorbance by formulations at different concentrations. According to these results, it can be speculated that the antioxidant properties were solely caused by cashew plant extract which was incorporated in the ointment base.

Keywords: Cashew plant leaf, Natural products, Herbal ointment, Antioxidant activity, DPPH free radical scavenging activity

Introduction:

Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and aliments. Nature has bestowed our country with an enormous wealth of medicinal plants; therefore, India has after been referred to as the medicinal garden of the world. The other main source of medicinal plants is from cultivation. The cultivated material is definitely more appropriate for use in the production of drugs [1]· Along with other dosage forms herbal drugs are also available in the form of ointment which is semisolid preparation used topically for several purpose as protectants antiseptic, anti healing, emollient, keratolytic &astringents [2]. The development of new drugs relying purely on modern technology appears to be reaching something of a limit. In developing new drugs, the pharmaceutical industry has tended to adopt high-throughput synthesis and combinatorial chemistry-based drug development since the 1980s; however, the efforts made in this direction have not resulted in the expected drug productivity. Some large pharmaceutical companies are facing great challenges to develop new products. Over the past dozen years, increasing attention has accordingly been paid to natural products in the search for novel drugs in combination with new technology, such as high-throughput selection [3]. Natural products have a wide range of diversity of multi-dimensional chemical structures; in the meantime, the utility of natural products as biological function modifiers has also won considerable attention [4]. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Antioxidant activity in traditionally used plant species is a method of scientific validation of the medicinal plant use by Indigenous Peoples. Plant species that are used traditionally for multiple symptoms could indicate a high level of antioxidant activity [5].

Materials and Method

Animal Used: Healthy adults Albino Wistar Rats strain weighing 180-250 gram were used for the study.

Extraction of plant: Solvent extraction of selected plant i.e. Anacardium occidentale leaves was performed using soxhlet extractor. Air-dried powdered of Anacardium occidentale leaves marc was macerated with ethanol for 24 h (three times) to obtain the ehanol extract. The obtained extract concentrated using rotary vaccum evaporator [6].

Isolation of compounds from extract of Anacardium occidentale leaves: The crude extract obtained was suspended in distilled water in a separating funnel (5 L) and in turn partitioned with n-hexane, dichloromethane, ethyl acetate and n-butanol (BuOH). This yielded four solvent fractions, Column Chromatography using silica gel (60-120 mesh) as the stationary phase was used to fractionate ethyl acetate fraction. The column was eluted with gradient elution with n-hexane: ethyl acetate starting with 10% up to 100% ethyl acetate. This was followed with an increasing gradient of methanol from 10% in ethyl acetate up to 100% methanol.

Fractions (20 mL each) collected were analysed on TLC plates using Hexane/EtOAc (5:5). This afforded five fractions (fr1 to fr5). Fraction 5 showed two spots on TLC plate which were fractionate on silica gel column using chloroform: methanol gradient elution, chloroform (100%) was followed by an increased gradient of methanol up to 50%. Test tube fractions collected were analysed on TLC plate using chloroform: methanol (9:1) solvent system. Three fractions were obtained. Open column chromatography using Sephadex LH-20 as stationary phase was used to purify the isolated compound showed single spot on TLC [7-8].

Preparation of herbal ointment containing isolated compound: The herbal ointment formulation of isolated compound to ointment base mixture. First the ointment base was weighed accurately, which was placed in evaporating dish on water bath. After melting of hard paraffin remaining ingredients (Wool fat (0.5 g), Cetostearyl alcohol (0.5 g) and Yellow soft paraffin (8.0 g) were added and stirred gently to aid melting and mixing homogeneously followed by cooling of ointment base. Now for second step, herbal ointment was prepared by mixing accurately weighed extract of Anacardium occidentale leaves and isolated compound to the ointment base by levigation method to prepare a smooth paste with two or three times its weight of base, gradually incorporating more base until to form homogeneous ointment, finally transferred in a suitable container. The plant extract gel was finally transferred in aluminium collapsible tube and labeled [9].

AO1: Herbal ointment containing 20% Anacardium occidentale leaves extract

AO2: Herbal ointment containing 1% isolated compound

AO3: Herbal ointment containing 2% isolated compound

Evaluation parameters:

Colour and Odour: Physical parameters like colour and odour were examined by visual examination.

Consistency: Smooth and no greediness is observed.

pH: PH of prepared herbal ointment was measured by using digital PH meter. The solution of ointment was prepared by using 100ml of distilled water and set aside for 2hrs. PH was determined in triplicate for the solution and average value was calculated.

Spreadability: The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as spreadability.

Extrudability: The formulation was filled in collapsible tube container. The extrudability was determined in terms of weight of ointment required to extrude 0.5cm of ribbon of ointment in 10 seconds.

Diffusion study: The diffusion study was carried out by preparing agar nutrient medium. A hole board at the centre of medium and ointment was by placed in it. The time taken by ointment to get diffused through was noted. (After 60 minutes)

LOD: LOD was determined by placing the formulation in Petri-dish on water bath and dried for the temperature 105°C.

Solubility: Soluble in boiling water & Miscible with alcohol, ether, chloroform.

Wash ability: Formulation was applied on the skin and then ease extends of washing with water was checked.

Non-irritancy Test: Herbal ointment prepared was applied to the skin of human being and observed for the effect.

Antioxidant activity by DPPH free radical scavenging activity: The free radical scavenging activities of the herbal ointment on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) will be estimated by the method described by (Leitao et al., 2002). 2.0 ml of a methanol solution of the sample (extract/control) at different concentration (50–250µg/ml) will be mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 min reaction period at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer.

Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$I\% = (1 – A \text{ sample}/A \text{ blank}) \times 100$

where A blank is the absorbance of the control reaction (containing all reagents except the test material). Extract concentration providing 50% inhibition (IC50) will be calculated from the graph plotted inhibition percentage against extract concentration [10].

Results and Discussion

Extraction process of plant metabolites in the crude drugs was done by Soxhlet extraction process. Extractive values are useful for the evaluation of nature of the active phytoconstituents present in the drug especially when the constituents of a drug cannot be readily estimated by any other means. The coarse powders of the leaves were subjected to maceration solvent extraction using ethanol. Isolation of compounds from extract of Anacardium occidentale leaves was done by column chromatography. The various formulation herbal ointments AO1 – AO3 were prepared using Anacardium occidentale leaves extract and isolated compound. The prepared ointment AO1 – AO3 formulation were evaluated using parameters like; physical appearance, ph determination, extrudability determination, viscosity determination, spreadability, homogeneity, grittiness.

Table 1: Physical parameters of herbal ointment formulation

Parameters	**AO1**	**AO2**	**AO3**
Colours	Light Green colour	Green colour	Pale yellow colour
Appearance	Translucent	Transparent	Translucent
Odour	Pleasant odour	Pleasant odour	Define odour
Feel of application	Smooth	Smooth	Smooth
Spreadability (g.cm/sec)	9.1	10.3	10.9
Consistency	Good	Fairly good	Good
pH	6.81	6.84	6.52
Viscosity (cps)	0.92	0.99	0.94
Extrudability	Poor	Good	Good

Anti-oxidant Activity: Analysis of the free radical scavenging activities of the selected Anacardium occidentale leaves and isolated compound revealed a concentration dependent free radical scavenging activity resulting from reduction of DPPH, NO radical to non-radical form. The scavenging activity of Ascorbic acid, a known antioxidant used as positive control, was however higher.

DPPH radical is considered to be a model for a lipophilic radical. A chain in lipophilic radicals was intiated by the lipid autoxidation. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capacity of DPPH was determined by the decrease in its absorbance at 517nm, which is induced by antioxidant. Positive DPPH test suggests that the samples were free radical scavengers. The scavenging effect of l-Ascorbic acid, and plant extracts increased gradually with increase in concentration.

Nitric oxide plays an important role in various types of inflammatory processes in the body. In the present study Anacardium occidentale leaves and isolated compound checked for its inhibitory effect on Nitric oxide production. Nitric oxide radical generated for sodium nitroprusside at physiological pH was found to be inhibited by the extracts. The herbal ointment formulation at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to other extract. Results revealed that all the tested extract showed the percentage of inhibition in a dose dependent manner. The herbal ointment at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity.

Table 2: Effect of herbal ointment containing Anacardium occidentale leaves extract and isolated compound on DPPH radical scavenging model

Concentration	50µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml
Ascorbic acid	42.36 ± 1.31	53.23 ±0.92	66.09±1.64	82.18±0.85	91.92±1.5
AO1	32.11± 1.1	45.71±1.2	51.21±1.1	62.23±1.2	78.03±1.3
AO2	22.01± 1.3	38.11±1.3	42.13±1.3	54.11±1.2	70.12±1.2
AO3	30.01± 1.3	42.53±1.1	48.11±1.3	59.03±1.1	75.01±1.1

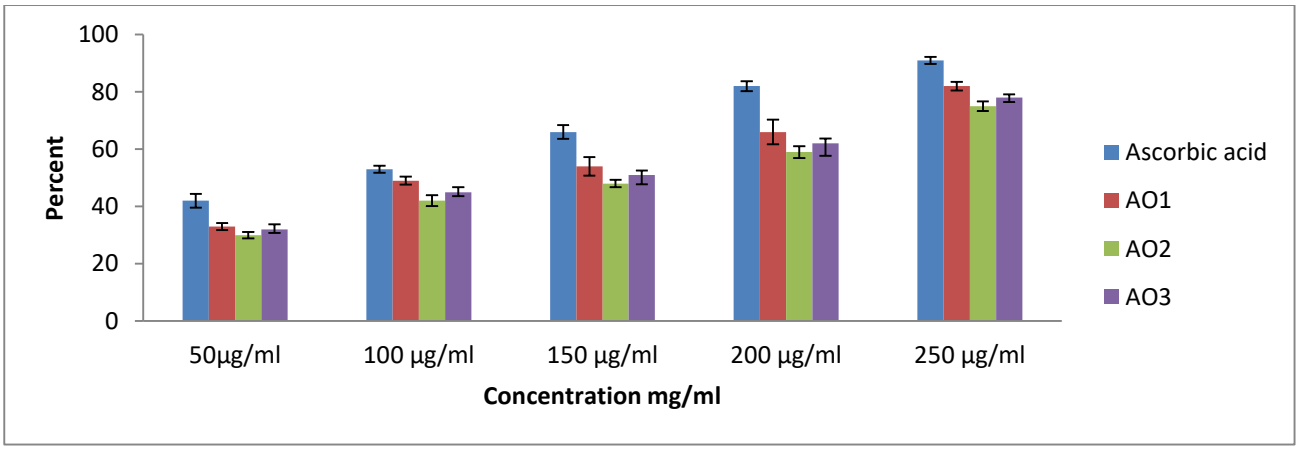

Figure 1: Results of Anacardium occidentale leaves extract and isolated compound on DPPH radical scavenging model

Conclusion: From the ancient time plants are used for their various medicinal properties like, anti healing, ant diuretic, skin eruption, impotence, etc. Thus this ointment could become a media to use these medicinal properties effectively and easily as a simple dosage form. The herbal ointment had acceptable physicochemical properties based on macroscopic observation, pH, and viscosity measurements. These loaded cream also had satisfactory antioxidant activity, which can be regarded as an effective and economical skincare product for topical uses. Natural Remedies are more acceptable as they are safer with fewer side effects than synthetic once. So, a herbal formulation is non toxic, safe, effective and improves Patient Compliance as it contains herbal ingredients. From the ancient time semisolid formulations is used for their various medicinal properties like, anti healing, anti diuretic, skin eruption etc. Thus this ointment could become a media to use these medicinal properties effectively and easily as a simple dosage form. In present study the herbal ointment is showing better results than standard marketed formulation.

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