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Pharmacological studies of *Azadirachta indica* leaves extracts

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Abstract

The present study was undertaken to explore the anticarcinogenic, anti-bacterial and anti-oxidant screening of the hydro-methanolic leaves extract of *Azardirchta indica using* mouse skin papilloma model, disc diffusion and DPPH methods. In phytochemical screening, *Azardirchta indica extract* showed presence of secondary metabolites such as carbohydrate, tannins etc. In anticarcinogenic studies, the Tumour incidence was 100% in control group which was reduced in treated group and remains 60.0%. in Control group the papilloma appeared early i.e on 5th week which was increased week wise and finally reaches upto 26 in numbers, whereas it was 9 on treated group by the same time. Results denote the anticarcinogenic, antibacterial and antioxidant activities of the *Azardirchta indica leaves* extracts. The *Azardirchta indica leaves* extracts possessed potent hydroxyl radical scavenging activity against the positive control Ascorbic acid. The antibacterial activities were also observed against the test organisms.

Keywords: *Azadirachta indica*; Anticarcinogenic Antibacterial; Antioxidant

1. Introduction

Plants and their secondary metabolite constituents have a long history of use in certain systems of traditional medicine and use of herbal medicine in developed countries have expanded sharply in the twentieth century. Neem *(Azadirachta indica)*, a member of the Meliaceae family is a fast growing tropical evergreen tree with a highly branched and stout, solid stem. Because of its tremendous therapeutic, domestic, agricultural and ethnomedicinal significance and its proximity with human culture and civilization, neem has been called “the wonder tree” and “nature’s drug store.” The plant product or natural products show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways. The therapeutics role of number of plants in diseases management is still being enthusiastically researched due to their less side effect and affordable properties. It has been accepted that drugs based on allopathy are expensive and also exhibit toxic effect on normal tissues and on various biological activities. It is a largely accepted fact that numerous pharmacologically active drugs are derived from natural resources including medicinal plants [1, 2]. Islamic perspective also confirms the herbs role in diseases management and recommended various plants/fruits in the diseases cure [3]. Neem ingredients are applied in Ayurveda, Unani, Homeopathy, and modern medicine for the treatment of many infectious, metabolic, or cancer diseases [4, 5]. Different types of preparation based on plants or their constituents are very popular in many countries in diseases management. *Azadirachtaindica* has complex of various constituents including nimbin, nimbidin, nimbolide, and limonoids and such types of ingredients play role in diseases management through modulation of various genetic pathways and other activities. Quercetin and ß-sitosterol were first polyphenolic flavonoids purified from fresh leaves of neem and were known to have antifungal and antibacterial activities [6]. Numerous biological and pharmacological activities have been reported including antibacterial [7], antifungal [8], and anti-inflammatory. Earlier investigators have confirmed their role as anti-inflammatory, antiarthritic, antipyretic, hypoglycemic, antigastric ulcer, antifungal, antibacterial, and antitumour activities [9–12] and it is summarized the

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various therapeutics role of neem [13]. Therefore the chemopreventive/ anticancer effects of neem extracts have been studied.

2. Material and methods

2.1 Plant collection and identification

The leaves of *Azardirchta indica were* procured from Satna (Madhya Pradesh) and authenticated by Botanist, Manoj Tripathi of DRI, Chitrkoot Madhya Pradesh (India). The ethical approval was obtained before the experiments. All the materials and reagents used for the study were from CDH, Renchem and Hi- Media Ltd., India.

2.2 Preparation of *Azardicta indica* extract

The collected leaves were dried in shade and grinded with mechanical grinder. About 50g powder poured in separating funnel with 50% methanol for 48hrs. The collected residues were kept at 50°C in water bath to concentrate it and finally transfer into the Hot Air Oven to dry it. About 5.8 g crude extract was prepared (Yield= 19%) and used for the furtherstudies.

2.3 Phytochemical screening

Phytochemicals are nonnutritive plant chemicals that contain protective, disease- preventing compounds. Standard screening test were carried out for various plant constituents. Hydro-methanolic crude extract were screened for presence or absence of secondary metabolites such as alkaloids, tannins, steroids, phenols, flavonoids, saponins and Phlobatannins etc. using standard procedures to identify the constituents as described by us (Agrawal,2021) 19.

2.4 Experimental design for Skin Carcinogenesis

The dorsal skin on the mice back was shaved 1 day before the experiment commenced .Only animals in the hair cycle resting phase were chosen. Two stage protocol initiated by a single topical application of a carcinogen (7, 12 - dimethylbenz (a) anthracene (DMBA) and then followed by a promoter (croton oil) twice in a week were employed as per Berenblum,1975 **21** as standardized by Agrawal et al, 2009**)20** used to induce tumours. Animals were randomly allocated into 7 groups of comprising six mice each. The treatment was provided topically to the shaved area.

2.4.1 Treatment Groups

- Group 1 (Untreated control): No treatment
- Group 2 (Vehicle control): Twice a week administration of 100 µl acetone up to 16 weeks
- Group 3 (DMBA Alone): - Single administration of 100 µg DMBA dissolved in 100 µl acetone.
- Group 4 (Croton Oil Alone): - Twice a week application to skin of 1 % Croton oil up to 16 week.
- Group 5 (*Azardirichta indica leaves* Extract Alone): - Twice a week application to skin at the dose of 500 mg/kg b. wt up to 16 week.
- Group 6 (DMBA + Croton Oil): - Single application to skin of 100 µg DMBA in 100 µl acetone afterwards 1 % croton oil was applied on skin twice a week up to 16 week.
- Group 7 (DMBA + *Azardirichta indica leaves*+ Croton Oil): -

Single application of100 µg DMBA in 100 µl acetone afterwards the 100 µl dose of 1 % Croton oil. Afterwards Twice a week application to skin at the dose of 500 mg/kg b. wt of Neem extract up to 16 week.

All animals groups were observation for gross and microscopic skin changes weekly during the 16 weeks of experimentation period. All mice were weighed and examined for skin papillomas and results were recorded.

Antibacterial activities of hydro-methanolic extract from leaves of *Azardirithtca indica* was investigated using the Disk diffusion method as suggested by Kerby-Bauer Disk Diffusion Susceptibility test. Following gram negative and gram positive bacterial strain i.e., *Escherichia coli,* and *Bacillus subtilis were* used for the Antibacterial activities which were received from stock culture of our laboratory. Nutrient agar broth media were used for the antibacterial activities. Nutrient Agar media prepared and poured in Petri- plates after solidifying swab of the bacterial cultures on the plates and allowed for incubation at 37^0C for 24 hrs...Measurement of Zone of Inhibition (In mm). Sterile nutrient agar plates were inoculated with the test culture by surface spreading using sterile wire loops and each bacterium evenly spread on the entire surface of the plate to obtain uniformity of the inoculums 4 different concentrations of crude extract were prepared (100%, 50%, 25%,12.5%,) and were used for antibacterial analysis using agar disk diffusion methods.. Paper

disks were made in each of the plate with a sterile 2.0 mm diameter .Each of the four disk was soaked in a given concentration of the extract mixed with plane sterile agar. The plates were then incubated at 37°c for 24 hours. The diameters of zones of inhibition were measured using a meter rule and the mean value for each organism was recorded.

2.5 DPPH radical scavenging assay

The radical scavenging activity of *A. indica* extracts against the DPPH radical was determined by the standard method.Determination procedures were as follow: 1 mL of 6 × 10−5 M DPPH radical solution (prepared daily) was mixed with 33.33 μL of methanolic solutions of *A. indica extracts* (maximum dissolved concentration). After 30 min incubation for at 37 °C, absorbance decrease of the mixture was monitored at 515 nm. During reduction by the antioxidant, the solution colour changed from violet to yellow pale. DPPH radicals have an absorption maximum at 515 nm. Blank samples with 33.33 μL of methanol in the above DPPH radical solution were prepared and measured daily at same wavelength (*Ab*). The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula. Inhibition rate % = Ab -As ×100 Ab .The 50% inhibitory concentration (IC50) was expressed as the quantity of the extracts to react with a half of DPPH radicals.

3. Results

Table 1 Phytochemical present in the hydromethanolic extract of *Azardiricta indica leaves* extract

Sr. No.	Phytochemical Test	Hydromethanolic Extract
I.	Test for Carbohydrates and reducing sugars	
A	Fehling's Test	+
II	Test for Phenolic compound's	
A	Ferric Chloride test	-
III	Test for Tannins	
A	Lead Acetate test	+
IV	Test for Phytosterols	
A	Salkowski Test	_
V	Test for Proteins	
A	Biuret Test	_
VI	Test for Saponins	
A	Foam Test	-
VII	Test for Flavanoid	
A	Lead Acetate Test	-

+ denotes Present and – denotes Absent

Table 2 Cumulative No. of Papilloma in the animals treated with *Azardicaindica* leaves extract

Groups	Dose	Time of 1st appearance of Papilloma	Cumulative No. of Papilloma	Tumour yield	Tumour incidence
Vehicle alone	100µl/animal	–	–	–	–
DMBA alone	100µg/animal	–	–	–	–
Croton Oil alone	1% per animal	–	–	–	–
Azardicta indica leaves Extract alone	500 mg/kg per animal	–	–	–	–
DMBA + CO (Control)	100µg + 1% per animal	58th Day	47	47/6 (7.8)	6/6 (100%)
DMBA+CO+ *Azardicta indica leaves* extract.	100µg + 1% + 500mg/animal	61th Day	11	11/5 (2..2	4/6 (60 %)

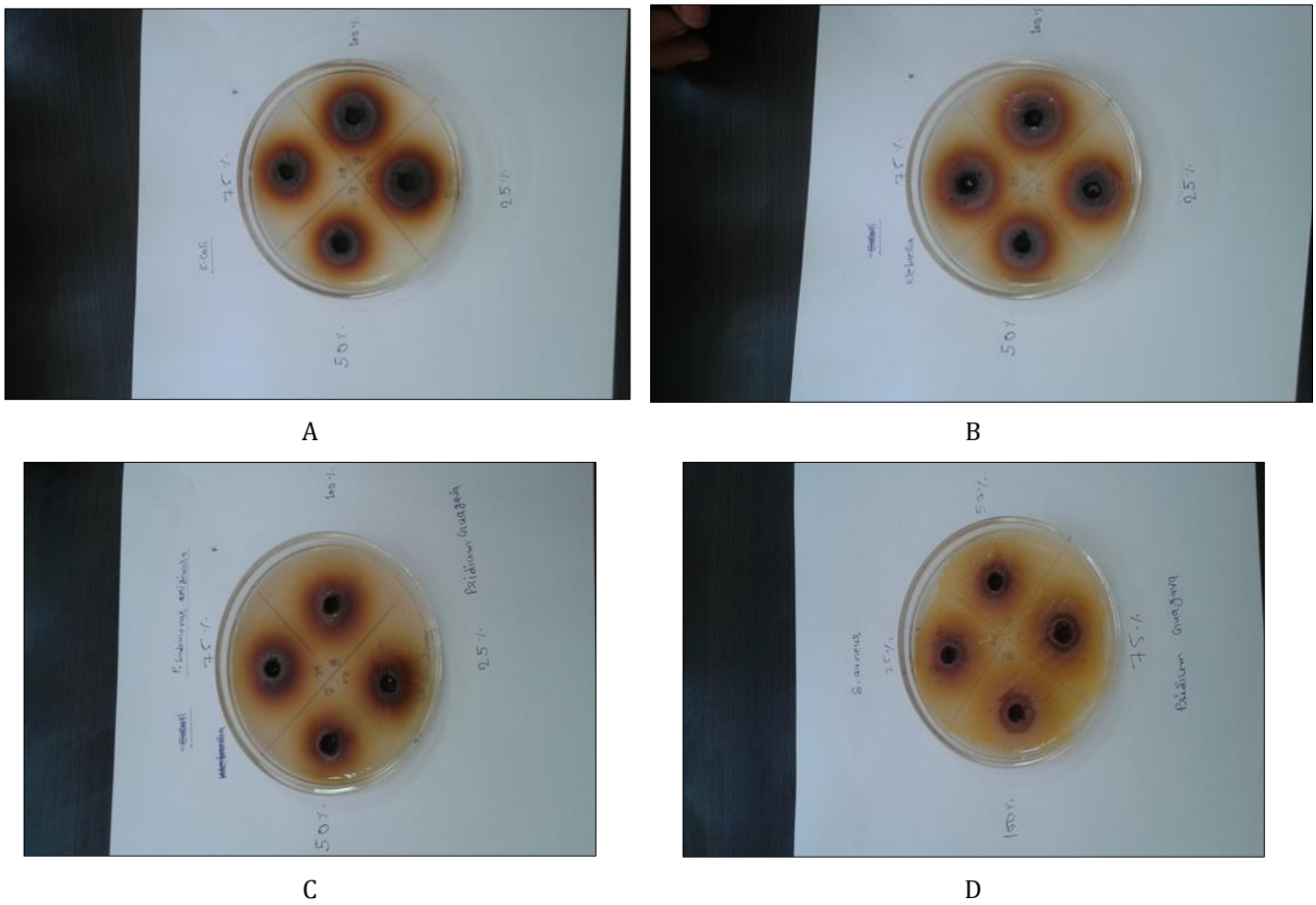
Tables 3 Antibacterial activity of *Azardicaindica* against bacterial strains

Name of microorganisms	% Concentration of Extract [zone of inhibition(mm)]			
	25	50	75	100
B. subtilis	10	12	15	18
E. Coli	9	12	13	18

Table 4 *In vitro* antioxidant activity of 50% methanolic *Azardichta indica* extracts Vs Ascorbic acid (standard)

Sr. No.	Concentration of ascorbic acid (µg)	% TBARS inhibition	Concentration of *Azardicaindica*(µg)	% TBARS inhibition
1	50	25	10	8.2
2	100	73	20	52.7
3	150	165	30	24.02
4	200	96	40	36.38
5	250	55	50	46.6
6	300	144	60	46.3
7	350	145	70	55.2
8	400	171	80	66.2
9	450	409	90	73.3
10	500	270	100	104.5

IC_{50} = Concentration at which % inhibition of TBARS is 50%.



A B C D

Figure 1 A, B, C and D showed the zone of inhibition

4. Results and Discussion

The present study showed anticarcinogrnic, antibacterial and antioxidant activity of the crude hydromethanolic ieaves extract of *Azardichta* indica. For Anticarcinogenic study: in (DMBA + Croton oil) the mean number of papillomas and Tumour incidence was 100% in control group which was reduced in treated group and remains 60.0%. (Table no. 2). Antibacterial activity: 50% methanolic extract of leaves of *Azardiricta indica* at the different concentration i.e. 25%, 50%, 75%, 100% exhibited antibacterial against *Bacillus subtilis* and E*. coli (Table No.3)* . The in vitro antioxidant activity of Azardriicta indica leaves was tested in various concentrations against Ascorbic acid as standard. Percentage of TBARS was calculated for both Ascorbic acid and *Azardicaindica* extract, with the help of formula, for a comparative study (Table No.4).

5. Conclusion

Neem (*Azadirachta indica*), a member of the Meliaceae family, has therapeutics implication in the diseases prevention and treatment. But the exact molecular mechanism in the prevention of pathogenesis is not understood entirely. It is considered that *Azadirachta indica* shows therapeutic role due to the rich source of antioxidant and other valuable active compounds such as azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, salannin, and quercetin. Neem (*Azadirachtaindica*) plants parts shows antimicrobial role through inhibitory effect on microbial growth/potentiality of cell wall breakdown. It has been reported that Neem ingredient shows effective role in the management of cancer through the regulation of cell signaling pathways and reported to prevent the tumour development [14-19]Azadirachtin, a complex tetranortriterpenoid limonoid present in seeds, is the key constituent responsible for both antifeedant and toxic effects in insects [23]. Results suggest that the ethanol extract of neem leaves showed *in vitro* antibacterial activity against both *Staphylococcus aureus* and MRSA with greatest zones of inhibition noted at 100% concentration [24]. Neem plays role as free radical scavenging properties due to rich source of antioxidant. Azadirachtin and nimbolide showed concentration-dependent antiradical scavenging activity and reductive potential [25]. The results indicated that dietary use of extracts from various parts of *A. indica* may play a promising role in future

drug discovery and development programs as far as chemoprevention of cancer is concerned. Most of the ethnomedicinal and early studies on neem with respect to its anticancer properties suffered from lack of credible mechanistic principles.

Compliance with ethical standards

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