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Research Article

IN-VITRO ASSESSMENT OF ANTIBACTERIAL PROPERTIES OF SOME WILD HERBACEOUS SPECIES OF EUPHORBIACEAE AGAINST PATHOGENIC STRAINS

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ABSTRACT

Increasing bacterial resistance is prompting resurgence in research of the antimicrobial role of herbs against resistant strains. A vast number of plants have been recognized as valuable resources of nature antimicrobial compounds. In the present study antibacterial properties of leaf extracts of four wild herbaceous species of family Euphorbiaceae i.e. *Euphorbia hirta, Euphorbia microphylla, Croton bonplandianum* and *Phyllanthus niruri* were evaluated in vitro against two pathogenic bacteria *E. coli* - ATCC10536 and *Staphylococcus aureus* - ATCC 25923. Leaf extracts were prepared in three solvents i.e. Ethanol, Methanol and Hot water, which toxicity in graded concentrations of 25%, 50%, 75% and 100% crude extracts have been assessed *in-vitro* by measuring zone of inhibition for both pathogen applying well diffusion method, comparing with the toxicity of standard antibiotics. Extracts of *Phyllanthus niruri* and *Croton bonplandianum* showed more significant result rather than *Euphorbia microphylla,* and *Euphorbia hirta* against both pathogens, while better in E. coli than S. aureus. Ethanol extracts was found most effective for inhibiting bacterial growth, whereas the ZOI in case of 100% methanol extracts of *Phyllanthus niruri*, showed superior phytotoxic activity; however, based on the size of ZOI antibacterial properties was found in corresponding manner – Antibiotics > *Phyllanthus niruri* > *Croton bonplandianum* > *Euphorbia hirta* > *Euphorbia microphylla.* Wild as well as herbaceous plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat.

INTRODUCTION

Nature has been a source of medical agents for thousand of year and an impressive number of modern drugs have been isolated from natural sources; many of this isolation were based on the uses of the agents in traditional medicine (Cragg and Newman, 2001). Medicinal plants are the richest bio-resources for the discovery of novel bioactive compounds. The ancient Egyptians were familiar with many medicinal herbs and were aware of their usefulness in the treatment of various diseases (Abu-Shanab *et al*., 2004). Plants as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. According to the World Health Organization plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. Over 50% of all modern clinical drugs are of natural product origin (Kirbag *et al*., 2009).

In herbal medicines, crude plant extracts in the form of infusion, decoction, tincture or herbal extract are traditionally used by the population for the treatment of diseases, including infectious diseases. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents (Barnes *et al*., 2007). Plant-derived products contain a great diversity of phytochemicals such as phenolic acids, flavonoids, tannins, lignin and other small compounds (Cowan, 1999). These compounds possess numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic and vasodilatory activities. The expanding bacterial resistance to antibiotics has become a growing concern worldwide (Gardam, 2000). Plant extracts are used in herbal medicine for treatment of various human diseases (Begun *et al*., 1995; and Ross, 1999). Plants toxicity and biocidal activity has been widely investigated (Saksena and Tripathi, 1985; Begun *et al*.,

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1995; Deena and Thoppil, 2000; Sefidkon, 2002 and Kasali *et al.*, 2004).

A wide range of phytochemicals present in plants are known to inhibit bacterial pathogens (Cowan, 1999; Medina *et al*., 2005; Romero *et al*., 2005). Successful determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone, and methanol are often used to extract bioactive compounds (Eloff, 1998). Ethanol, however, is the most commonly used organic solvent by herbal medicine manufactures because the finished products can be safely used internally by consumers of herbal extracts (Low Dog, 2009). Additionally, the bioactivity of plant extracts depends on the water and ethanol concentration used in the extraction process (Ganora, 2008).

In view these facts the present course of work was planned to assess the antifungal properties of locally available herbaceous plants belong to Euphorbiaceae family – *Euphorbia hirta, Euphorbia microphylla, Croton borplandianum* and *Phyllanthus niruri.*

MATERIAL AND METHODS

Herbaceous wild plants of the family Euphorbeaceae was collected from local and identified with help of taxonomic key. Out of identified plants only four herbs were selected for extraction and toxicity assessment during present investigation.

Euphorbia hirta: As native to India is a pan-tropical weed, found especially on roadsides and wasteland. It has been traditionally used for female disorders but is now more important in treating respiratory ailments, especially cough, bronchitis and asthma, worm infestation in children and for dysentery, gonorrhoea, jaundice, pimples, digestive problems and tumours. The fresh milky latex is applied to wounds and warts and the root is used in sprains and inflammation, miscarriage, epilepsy, maggots in wounds and irregular growth of teeth.

Euphorbia microphylla: As an annual herb with pan-tropic distribution, this is mainly found in waste lands, along roadsides and wall sides under humid conditions. Its stem is slender, smooth, and reddish in colour and profusely branched. According to Charak the soup of Dhudhika is beneficial in diarrhea and painful bleeding of piles. The extract of this plant is applied for the cure of ringworms.

Croton borplandianum: As a wild weed, it is locally called Ban Tulsi / Kala Bhangra / Mirchaiya Jhaar. It is an annual herb plant grows mainly as a bush, profoundly grows around the canal, river banks, big drainage, waste lands, etc. Common names include Jungli Tulsi and Kala bhangra. The plant extract is also effective against green gram leaf curl disease (Aslam *et al*., 2006). Plant of used it as both fuel and detergent. The leaf extract shows antiviral activity against tomato spotted with virus coepia, leaf paste is applied for the skin diseases.

Phyllanthus niruri: Usually occurring as a winter weed found throughout the hotter parts. It is common kharif weed found in both cultivated fields and wastelands, commonly known as Stone breaker / Halmeri / Bhumy amla. It has shown clinical efficacy in viral Hepatitis B. It is known for its liver healing properties, used for treatment of liver diseases, lipid lowering action, anti-diabetic action and antifungal action. Ayurveda recommends its use for bronchitis, leprosy, anemia, urinary discharge, asthma etc. Local people of Chhattisgarh and Jharkhand use it for the treatment of skin diseases, indigestion, cough, ulcers etc.

Preparation of extraction

Fresh leaves of selected plants material was washed under running tap water, air dried and an oven dried at 40^{o}c for 24hrs for crude extract separately under aseptic condition and stored in airtight bottles. Two separate samples of the plant material (1g of each sample) were air dried powdered and extracted with hot water (2ml), ethanol 100% (2 ml) and methanol 100% (2 ml) dissolved at aseptic condition. In this way, two different leaf extract were obtained: hot water extract (HE), ethanol extract (EE) and methanol extract (ME) of 25%, 50%, 75%, 100% concentration.

Assessment of toxic nature of herbaceous plant

To evaluate the in-vitro antibacterial efficacy of herbaceous plant leaf extract; bacteria were used as test system for the characterization of its toxin producing nature. Characterization of toxin producing plants *Euphorbia hirta, Euphorbia microphylla, Croton borplandianum* and *Phullanthus niruri* was done with the help of measurement ZOI.

Antibacterial bioassay

The antibacterial activity was screened by Zone Of Inhibition. Overnight cultures (at 37^{o}C for 24 h) of each bacterial strain (*E.*

coli - ATCC10536 and *Staphylococcus aureus* - ATCC 25923) were spread with glass rod on the surface of Nutrient Agar Plates (Table-1). The antimicrobial activity was screened using the cark borer well (4mm in diameter) diffusion method well were saturated with 50µl (1gm /2ml) of the leaf extract of *Euphorbia hirta, Euphorbia microphylla, Croton borplandianum* and *Phyllanthus niruri* and in under laminar air flow. Agar well diffusion method was used for determining antibacterial activity. Petri plates were prepared by pouring 25 ml of seeded nutrient agar and allowed to solidify. The plates were placed in incubator for 24 hrs. After 24hrs culture with spread on agar plates. A standard cork borer of 4mm diameter was used to cut uniform wells on the surface of the agar plate and 2ml extract of 3 dilutions prepared were introduced in wells. The plates were incubated at 37^{o}C for 24hrs. After incubation, the diameter of clear zones around each well is measured and compared against zone of inhibition produced by solution of known concentration of standard antibiotic Gentamycin (10 mg) and Kanamycin (10 mg).

Different concentrations (25%, 50%, 75% &100%) of extracts were used and results were observed.

RESUTS

Toxicity of crude extract of herbaceous wild plant against bacterial growth has been found variously *in-vitro* culture plate. In the present investigation, to evaluate the antibacterial efficacy of leaf extracts of *Euphorbia hirta*, *Euphorbia microphylla, Croton bonplandianum* and *Phyllanthus niruri* against the pathogenic bacterial strains (*E. coli* -ATCC10536 and *Staphylococcus aureus* - ATCC 25923) were observed by measuring the zone of inhibition. The inhibitions of zone at different concentration of the extracts against specific test organism were measured. Antibacterial properties of crude extracts were recorded when the zone of inhibition was found greater than 4mm. The extract restricted the growth of the bacteria on media around the well. The zones of inhibition as observed (PLATE-1, Fig. - i-x) were measured have been computed in TABLE- 1 to 5 and shown in figure. (PLATE-2, Fig. - I to IV).

Table 1 Antibacterial effect of leaf extracts of *Euphorbia hirta* on *E. coli* and *Staphylococccus aureus* and its comparison with standard antibiotics (Mean ± SD)

	Leaf Extracts & Standard Antibiotics	Concentrations	Zone of inhibition (mm.) (Mean ± SD)	
			***E. coli* ATCC10536**	***Staphylococcus aureus* ATCC 25923**
	Control	0%	00	00
Leaf Extract	Ethanol	25%	4.0±0.22	4.2±0.25
		50%	9.2±0.52	7.2±0.42
		75%	10.0±0.55	8.0±0.45
		100%	11.5±0.65	10.0±0.55
	Methanol	25%	4.5±0.35	4.0±0.32
		50%	6.5±0.65	6.2±0.36
		75%	9.0±0.56	8.4±0.47
		100%	10.0±0.55	9.0±0.54
	Hot Water	25%	5.5±0.65	5.0±0.56
		50%	6.2±0.54	8.5±0.55
		75%	10.5±0.66	10.0±0.58
		100%	11.0±0.72	10.5±0.60
Antibiotics	Gentamycin	10 mg. / disc	17.4±0.45	15.5±0.30
	Kanamycin	10 mg. / disc	20.00±0.35	16.5±0.73

Table 2 Antibacterial effect of leaf extracts of *Euphorbia microphylla* on *E. coli* & *Staphylococcus aureus* and its comparison with standard antibiotics (Mean± SD).

	Leaf Extracts & Standard Antibiotics	Concentrations	Zone of inhibition (mm.) (Mean ± SD)	
			***E. coli* ATCC10536**	***Staphylococcus aureus* ATCC 25923**
	Control	0%	00	00
Leaf Extracts	Ethanol	25%	3.8±0.15	3.0±0.13
		50%	5.5±0.45	5.4±0.42
		75%	7.0±0.62	6.2±0.25
		100%	8.5±0.62	7.4±0.66
	Methanol	25%	4.5±0.73	3.2±0.52
		50%	5.0±0.70	3.8±0.65
		75%	6.5±0.55	4.8±0.15
		100%	7.0±0.60	6.2±0.56
	Hot Water	25%	3.6±0.52	3.0±0.50
		50%	5.0±0.13	4.5±0.73
		75%	6.3±0.43	6.5±0.45
		100%	8.0±0.62	7.5±0.30
Antibiotics	Gentamycin	10 mg. / disc	17.4±0.45	15.5±0.30
	Kanamycin	10 mg. / disc	20.00±0.35	16.5±0.73

Table 3 Antibacterial effect of leaf extract of *Croton borplandianum* on *E. coli* & *Staphylococcus aureus* and its comparison with standard antibiotics (Mean ± SD).

	Leaf Extracts & Standard Antibiotics	Concentrations	Zone of inhibition (mm.) (Mean ± SD)	
			***E. coli* ATCC10536**	***Staphylococcus aureus* ATCC 25923**
	Control	0%	00	00
Leaf Extracts	Ethanol	25%	6.5±0.61	6.0±0.55
		50%	9.2±0.52	8.5±0.63
		75%	10.6±0.65	11.0±0.50
		100%	15.0±0.42	14.8±0.40
	Methanol	25%	6.4±0.60	6.2±0.58
		50%	8.6±0.65	8.0±0.50
		75%	10.0±0.51	10.5±0.63
		100%	12.5±0.65	12.0±0.70
	Hot Water	25%	6.0±0.55	6.4±0.60
		50%	8.4±0.64	7.5±0.45
		75%	9.0±0.55	8.2±0.62
		100%	11.0±0.73	10.5±0.70
Antibiotics	Gentamycin	10 mg. / disc	17.4±0.45	15.5±030
	Kanamycin	10 mg. / disc	20.00±0.35	16.5±0.73

Table 4 Antibacterial effect of leaf extract of *Phyllanthus niruri* on *E. Coli* & *Staphylococcus aureus* and its comparison with standard antibiotics (Mean ± SD).

	Leaf Extracts & Standard Antibiotics	Concentrations	Zone of inhibition (mm.) (Mean ± SD)	
			***E. coli* ATCC10536**	***Staphylococcus aureus* ATCC 25923**
	Control	0%	00	00
Leaf Extracts	Ethanol	25%	8.5±0.57	7.5±0.61
		50%	10.6±0.63	9.5±0.62
		75%	12.4±0.51	10.0±0.62
		100%	16.5±0.35	15.5±0.73
	Methanol	25%	6.5±0.50	6.0±0.55
		50%	8.3±0.40	8.6±0.45
		75%	10.2±0.65	9.6±0.52
		100%	12.5±0.73	11.0±0.60
	Hot Water	25%	7.5±0.61	6.0±0.50
		50%	10.6±0.42	8.4±0.45
		75%	12.5±0.73	11.0±0.50
		100%	13.0±0.15	12.0±0.70
Antibiotics	Gentamycin	10 mg. / disc	17.4±0.45	15.5±0.30
	Kanamycin	10 mg. / disc	20.00±0.35	16.5±0.73

Table 5 Antibacterial effect of leaf extracts of *Euphorbia hirta, Euphorbia microphylla, Croton borplandianum* & *Phyllanthus niruri* on *E. coli* & *Staphylococcus aureus* and its comparison with standard antibiotics 100% concentration (Mean ± SD).

	Leaf Extracts & Standard Antibiotics	Concentrations	Zone of inhibition (mm.)	
			E. coli	***Staphylococcus aureus***
Leaf Extracts	*Euphorbia hirta*	Ethanol	11.5±0.65	10.0±0.55
		Methanol	10.0±0.55	9.0±0.54
		Hot water	11.0±0.72	10.5±0.60
	Euphorbia microphylla	Ethanol	9.0±0.62	8.5±0.66
		Methanol	7.0±0.60	6.2±0.56
		Hot water	8.0±0.62	7.5±030
	Croton borplandianum	Ethanol	15.0±0.42	14.8±0.40
		Methanol	12.5±0.65	12.0±0.70
		Hot water	11.0±0.73	10.5±0.70
	Phyllanthus niruri	Ethanol	16.5±0.35	15.5±0.73
		Methanol	12.5±0.73	11.0±0.60
		Hot water	13.0±0.15	12.0±0.70
Antibiotics	Gentamycin	10 mg. / disc	17.4±0.45	15.5±0.30
	Kanamycin	10 mg. / disc	20.00±0.35	16.5±0.73

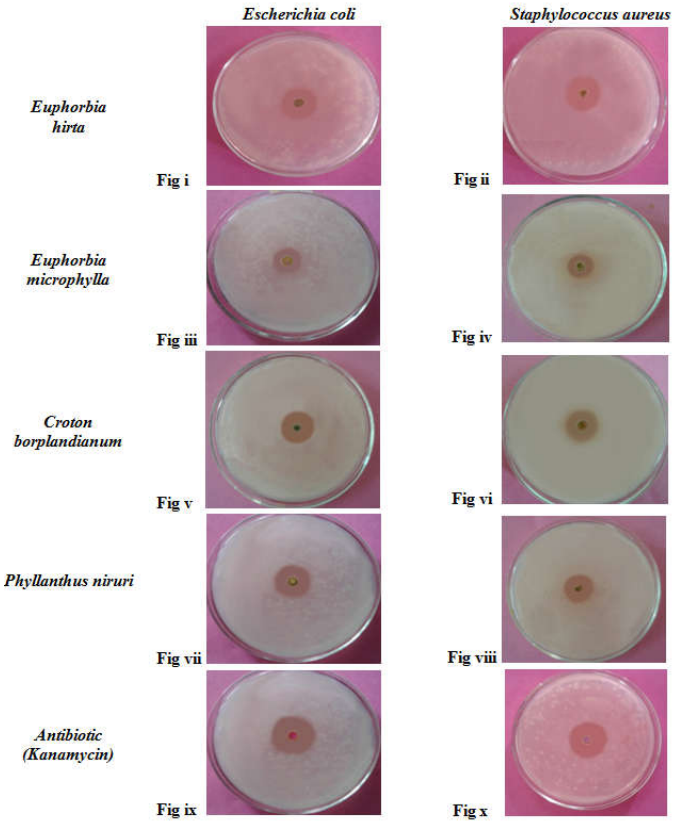

Plate 1 Fig i to x showing Zone of Inhibition in bacterial plates

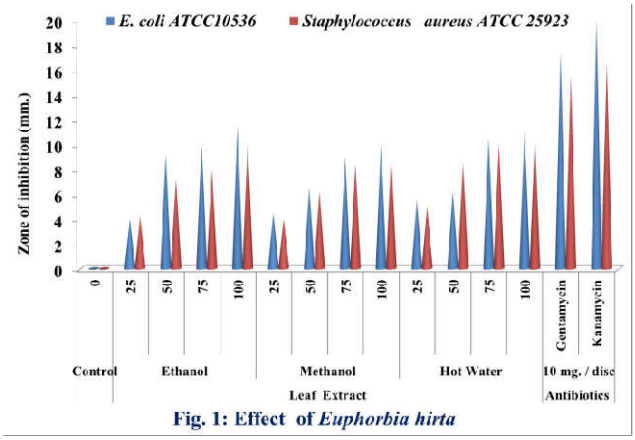

Fig. 1: Effect of *Euphorbia hirta*

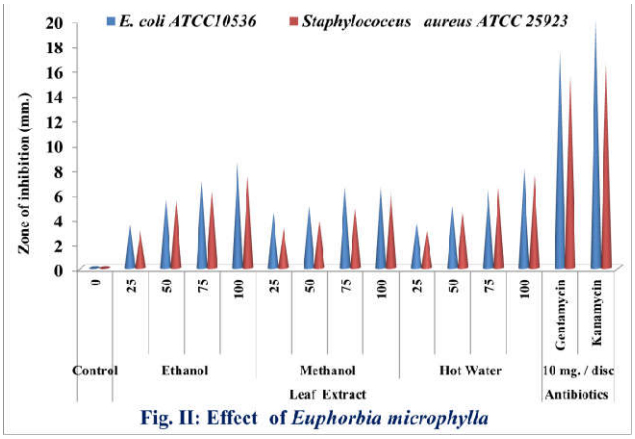

Fig. II: Effect of *Euphorbia microphylla*

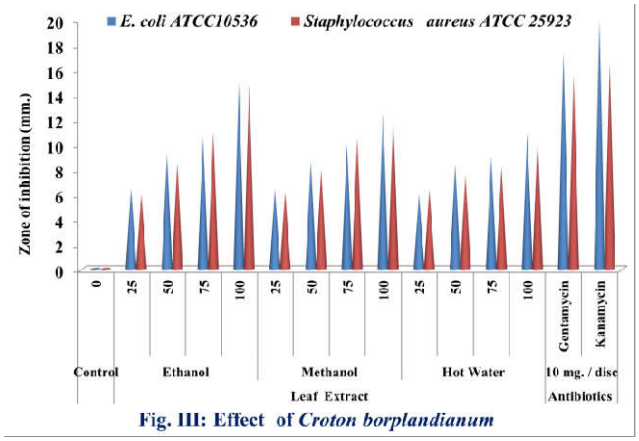

Fig. III: Effect of *Croton borplandianum*

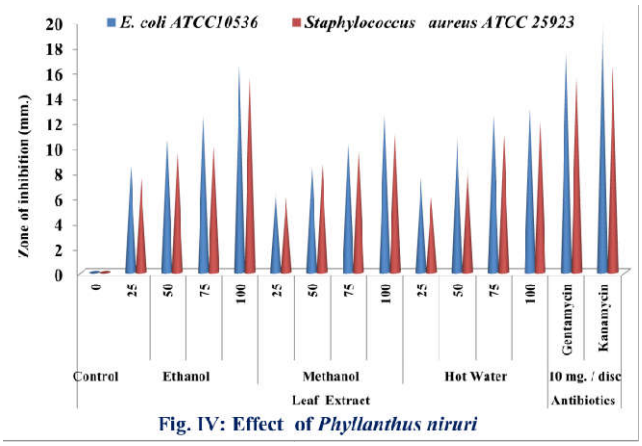

Fig. IV: Effect of *Phyllanthus niruri*

Plate 2 Fig. I to IV

DISCUSSION

Crude extract of *Euphorbia hirta* exhibited the toxicity against both the bacteria during present experiment. In case of ethanol extract against *E. coli,* ZOI was found significant in higher concentration i.e. 9.2mm in 50% , 10mm in 75% and 11.5mm in 100% ; effect of methanol extract was found lesser while the hot water extract affected more than methanol extract in 50%, 75% and 100% that ZOI was found 6.2mm, 10.5mm and 17.4mm respectively. More or less similar result was observed against *S. aureus*, however lesser than E. coli except of 50% concentration (TABLE-1).

Extracts of *Euphorbia microphylla* was showed toxicity against both the bacteria, but lesser than *Euphorbia hirta* (TABLE-2). Crude extract of *Croton bonplandianum* exhibited the toxicity against both the bacteria whereas ethanol extract against *E. coli* was noticed better inhibitory potential in higher concentrations while in case of *Staphylococcus aureus* it was found less (TABLE-4). *Phyllanthus niruri* was showed greater toxicity against both the bacteria during present experiment. In case of ethanol extract was found 8.5mm in 25%, 10.6mm in 50%, 12.4mm in 75% and 16.5mm in 100% concentration were as methanol extract found 6.5mm in 25%, 8.3mm in 50%, 10.2mm in 75% and 12.5mm in 100% concentration were as hot water extract found 7.5mm in 25%, 10.6mm in 50%, 12.5mm in 75% and 13mm in 100% concentration has been notice in case of bacteria *Staphylococcus aureus* ethanol extract was found 7.5mm in 25% , 9.5mm in 50% , 10mm in 75% and 15.5mm in 100% concentration were as methanol extract found 6mm in 25% , 8.6mm in 50% , 9.6mm in 75% and 11mm in

100% concentration were as hot water extract found 6mm in 25%, 8.4mm in 50%, 11mm in 75% and 12mm in 100% concentration, the maximum activity is shown by ethanol extract against *E. coli* bacteria.(TABLE-4).

Ethanol extracts all four herbs showed more significant toxicity against both pathogens, whereas in case of *E. coli*, it was noticed better effect. The zone of inhibition in 100% concentration / crude extracts was found nearer to the ZOI made by the effect of standard antibiotics. The findings of present investigation have correlated with the observation of other workers in case of another wild herbaceous plant (Abu-Shanad *et al.,* 2004; Krishnaraju *et al.*, 2005; Ganjewala *et al.*, 2009; Shrivastava *et al*., 2012).

CONCLUSION

On the basis of findings of the present study it can be concluded that crude extract of herbaceous wild plant have great potential against pathogenic bacteria, the results also revealed the fact that extract prepared using the ethanol extract possessed greater antibacterial activity. The present investigation also justifies the classified uses of the four herbaceous wild plants in the traditional system of medicine to treat various infectious diseases caused by microbes. Further chemical investigation may be carried out to isolate and identified the chemical constituents in the selected plants responsible for the antibaterial activity. The plant may be screened for other potential biological activities as well. Such screening of various natural organic compound of plant origin and identifying active agent is the need of the hour to meet the present therapeutic need and development of new drug.

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