Journal for Re Attach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 December; 6(1): 1550-1560

Pharmacological Screening Of Ethnomedicinal Plants In Rajasthan

Usha Kumari[1*], Santosh Joshi[2]

[1*,2]School of Basic and Applied Sciences, Raffles University, Neemrana, Alwar, Rajasthan–301705, India
***Email:** Ushakumari262019@gmail.com

ABSTRACT
Approximately 6% of the indigenous population of India resides in the state of Rajasthan. Ethnobotany is the study of the historical and traditional relationship between humans and the plant species in their environment. This relationship has been established over time through necessity, intuition, observation, and experimentation. The ethnobotany of India is considered one of the oldest in the world, and all traditional medical practices have their origins in this field. Rajasthan exhibits a high degree of cultural and biological diversity. WHO has acknowledged the significance of traditional medicine in various regions of the world. *ca.* 3800 plants species have been identified for their use in traditional herbal medicine. The accurate identification of these unrefined drugs in botanical nomenclature has not been conducted or is still a subject of controversy as diverse authors attribute different plant origins to several unrefined drugs. Furthermore, various complex ailments associated with energy, diabetes, and cognitive decline may be effectively treated through the use of herbal remedies, a feat that is typically unattainable through allopathic medicine. Nevertheless, there is a lack of organized record-keeping of this data. Medicinal plants exhibit a wide distribution across various habitats and landscapes.

Keywords: Ethnomedicine, Pharmacology, Antimicrobial, Immunomodulatory, Plants.

INTRODUCTION

The significance of medicinal plants for the well-being of individuals and communities is paramount. The therapeutic properties of these plants, which are of great economic value, are attributed to the existence of phytochemicals such as alkaloids, tannins, flavonoids, saponin, etc. that have distinct physiological effects on the human body (Edeoga et al., 2005). The identification of bioactive compounds in medicinal plants is a crucial aspect of phytochemical research on unrefined plant extracts and holds significance in terms of their plausible pharmacological impacts (Pascual et al., 2002). The inadequacies of current pharmaceuticals have prompted the exploration of novel pharmacotherapeutic agents derived from the study of medicinal plants (Cordell 1993). To advance the utilization of herbal medicines and assess their capabilities, there is a need for increased research on medicinal plants, particularly those employed in traditional medicine (Ali 2001; Nair et al., 2005).

The utilization of herbal medicines has gained increasing popularity among consumers who prefer natural methods for maintaining their health. These remedies have been employed since ancient times. Herbalism has been utilized as a form of healing in various major cultures throughout history (Zita, 1997). In recent decades, WHO has been promoting the utilization of primary indigenous medicinal plants as a means of treating various illnesses in developing nations. Ethnobotany is classified as a subdivision of ethnobiology. It is an interdisciplinary field of study that investigates the dynamic relationship between humans and plants. The term *ethno* refers to the study of people, while *botany* pertains to the scientific study of plants. Ethnobotany is a scientific field that investigates the intricate interconnection between the utilization of plants and the cultural practices of indigenous communities.

Ethnomedicine is a field of study that investigates the customary medical practices that pertain to the cultural understanding of health, disease, and illness, as well as the procedures involved in seeking healthcare and methods of healing. The term ethnomedicine pertains to the understanding and concepts surrounding healthcare and wellness (Singer M, 2011). When a plant is referred to as medicinal, it indicates that it contains one or more substances that have a beneficial effect on the physiology of ill mammals and have been historically utilized by humans for this purpose. According to Vedavathy S (2003), ethnomedicine is considered the precursor to the alternative medicinal systems. In numerous developing nations, a significant proportion of the populace relies on conventional practitioners and their collection of medicinal plants to meet their healthcare needs.

Ethnomedicinal research can yield a plethora of data concerning historical and contemporary connections between flora and indigenous cultures. Studies on the customary utilization and administration of indigenous flora have revealed the presence of comprehensive indigenous knowledge pertaining not only to the physical and chemical characteristics of numerous plant species, but also to the phenological and ecological attributes of domesticated species. Ethnobotanical research has been utilized in contemporary fields of study, including biodiversity prospecting and vegetation management, in addition to its conventional applications in economic botany and investigation of human cognition. The potential for ethnobotany to contribute to sustainable development and biodiversity conservation is a subject of great interest for researchers and practitioners alike. Ethnobotany is a field of study that intersects with various traditional scientific disciplines, including ethnomedicine and ethnopharmacology, among others. These subjects are interconnected and interdisciplinary in nature.

In recent times, there has been a renewed interest in plants as potential sources of medicinal compounds and unique molecules that can aid in the understanding of physiological and biological processes. There exist several reasons for this phenomenon. Primarily, developing nations hold a legitimate anticipation that their healthcare predicaments can be resolved through a rational scientific utilization of botanicals, many of which have been traditionally employed by the indigenous populace for several generations. Additionally, there exists a global movement towards the use of herbal medicines, based on the premise that they are less harmful to the human body compared to synthetic drugs. This movement is commonly referred to as the green revolution. Additionally, it is a well-established fact that numerous significant pharmaceuticals utilized in contemporary medicine were extracted from plants or possess lead compounds that originate from plants. Examples of drugs that fall under the categories of cardiac glycosides, alkaloids, and neuromuscular blockers include Vinblastine, Vincristine, Reserpine, etc. Molecules derived from plants, such as morphine, atropine, nicotine, etc. have been found to be highly useful in the identification of enzymes and the categorization of receptor systems (Elizabeth et al., 1978).

Table: Traditional uses and potential therapeutic properties of some ethnomedicinal plants in Rajasthan.

Species name	Family	Vernacular name	Part used	Pharmacological activity
Abrus precatorius L.	Fabaceae	*Chirmu Ratti*	Roots	Antitumor
Acanthospermum hispidum DC.	Asteraceae	*Dokanta*	Whole plant	Gastrointestinal
Achyranthes aspera L.	Amaranthaceae	*Andhi Jhara*	Roots	Antimicrobial
Aegle marmelos (L.) Corrêa	Rutaceae	*Billa*	Leaves	Anti-inflammatory
Barleria prionitis L.	Acanthaceae	*Danteli, Kala bans*	Whole plant	Anti-inflammatory
Butea monosperma (Lam.) Kuntze	Fabaceae	*Khankra, Sura*	Flowers	Antimicrobial
Calotropis procera (Aiton) W.T.Aiton	Apocynaceae	*Akra*	Root bark	Gastrointestinal
Capparis decidua (Forssk.) Edgew.	Capparaceae	*Kair*	Fruits	Antidiabetic
Chlorophytum tuberosum (Roxb.) Baker	Asparagaceae	*Dholi musali*	Tubers	Aphrodisiac
Curculigo orchioides Gaertn.	Hypoxidaceae	*Moosli*	Rhizome	Aphrodisiac
Dichrostachys cinerea (L.) Wight & Arn.	Fabaceae	*Goya-hair kolai*	Leaves	Gonorrhea
Dicliptera paniculata (Forssk.) I.Darbysh.	Acanthaceae	*Bhamwara Kakar*	Whole plant	Antimicrobial
Echinops echinatus Roxb.	Asteraceae	*Oont Kantilo*	Fruits	Anti-inflammatory
Enicostema axillare (Poir. ex Lam.) A.Raynal	Gentianaceae	*Nami*	Leaves	Antidiabetic
Euphorbia caducifolia Haines	Euphorbiaceae	*Danda Thore*	Latex	Antitumor
Gloriosa superba L.	Colchicaceae	*Kalihari*	Tubers	Immunostimulant
Helicteres isora L.	Malvaceae	*Anteri, Maror hali*	Fruits	Gastrointestinal
Holoptelea integrifolia (Roxb.) Planch.	Ulmaceae	*Sil, Kanjeri*	Bark	Antidiabetic
Hygrophila auriculata (Schumach.) Heine	Acanthaceae	*Talmakhana*	Leaves	Immunostimulant
Madhuca longifolia var. *latifolia* (Roxb.) A.Chev.	Sapotaceae	*Mori*	Bark	Gastrointestinal
Medicago sativa L.	Fabaceae	*Rizka*	Leaves	Anti-inflammatory
Senegalia catechu (L.f.) P.J.H.Hurter & Mabb.	Fabaceae	*Khair katha*	Bark	Antimicrobial
Solanum virginianum L.	Solanaceae	*Kateli, Bhurangni*	Whole plant	Anthelmintic
Soymida febrifuga (Roxb.) A.Juss.	Meliaceae	*Rohini*	Bark	Gastrointestinal
Terminalia anogeissiana Gere & Boatwr.	Combretaceae	*Dhawari*	Leaves	Antimicrobial
Tinospora cordifolia (Willd.) Hook.f. & Thomson	Menispermaceae	*Giloy*	Stem	Antidiabetic
Vachellia nilotica (L.) P.J.H.Hurter & Mabb.	Fabaceae	*Boriyo*	Gum	Immunodeficiency
Ziziphus nummularia (Burm.f.) Wight & Arn.	Rhamnaceae	*Jhari, Bordi*	Roots	Gastrointestinal

The current study focused on the selection of *Gloriosa superba* and *Hygrophila auriculata* plants based on their traditional significance in indigenous medicine. These plants were then evaluated for their ability to modulate the immune system. The plants in question have been conventionally employed by indigenous healers in diverse rural regions of Rajasthan to alleviate a range of ailments, including joint pain, fever, and skin conditions. Additionally, these plants are cited in ethnobotanical literature. The primary aim of this study is to explore the immunomodulatory properties of specific plants that hold traditional value, with the intention of identifying novel alternatives to existing immunomodulatory agents.

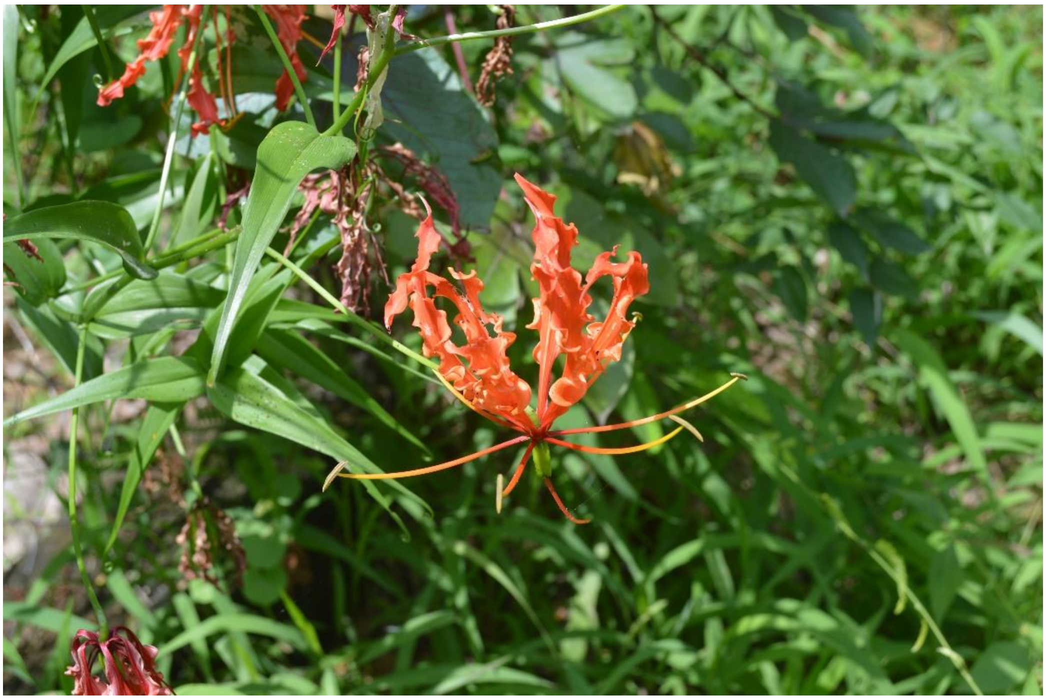

***Figure 1: Gloriosa superba* L.**

***Figure 2: Hygrophila auriculata* (Schumach.) Heine**

STUDY AREA

The southern region of Rajasthan is recognised as a tribal area, where the primary ethnic groups are Bhil, Damor, Garasia, Kalbelia, Kathodia, and Meena. The flora in the surrounding environment constitutes a fundamental aspect of their cultural heritage. The healthcare of these individuals is predominantly reliant on their customary healing practises, which are transmitted orally across generations (Schutes, 1992; Harshberger, 1986). The region is characterised by a tropical deciduous vegetation type, which includes important plant species such as *Anogeissus latifolia, Anogeissus pendula, Balanites aegyptiaca, Boswellia serrata, Diospyros melanoxylon, Madhuca indica, Tectona grandis, Terminalia arjuna*, among others. Several researchers have made noteworthy contributions to the field of ethnobotany in India (Schutes, 1992; Harshberger, 1986; Martin, 1983; Peters *et. al.*, 1989; King, 1869; King, 1870). Studies have been conducted in various regions of Rajasthan (Nathawat & Deshpande, 1960; Gupta, 1970; Vyas & Gupta, 1962; Raheja & Sen, 1964; Chopra *et. al.*, 1960; Gupta & Dutta, 1967; Gupta & Saxena, 1968; Bhandari, 1974; Dixit & Mishra, 1976; Srivastava, 1977). Despite the favourable conditions, there has been minimal research conducted on the ethnobotany and economic utilisation of local plant resources in Rajasthan. The region of Southern Rajasthan exhibits a relatively high diversity of plant species and is home to several indigenous communities with a significant cultural legacy. Vyas and Gupta (2014) documented several medicinal plant species found in the Alwar region. Raheja and Sen (2010) documented the botanical resources of Rajasthan in the context of its developmental potential. Chopra et al. (2014) discussed certain medicinal plants found in the arid zone of India. The useful plants of the regions were assigned vernacular names by Gupta and Dutta (1967). Gupta and Saxena (1968) conducted a study on *Salvadora oleoides* and *S. persica*, which are two significant plants known for their oil production. Bhandari (1974) provided a comprehensive description of the famine-resistant food sources found in the Marwar region. In their publication, Dixit and Mishra (1976) documented a number of medicinal plants that are relatively unknown within the Ajmer forest division. Srivastava (2016) provided a report on the forest resources present in the state of Rajasthan. Singh and Shetty (1977) conducted a survey on the natural resources present in the Rajasthan Desert. Paroda (1979) conducted a study on the utilisation of plant resources in the arid regions of India for industrial purposes. In a study conducted by Amalraj (1982), several plants from Rajasthan were identified as having potential for further investigation. Singh and Pandey (1982) documented the fiber-producing plant species found in Rajasthan. Joshi (1982) provided a report on the ethnobotanical practices of the Bhil tribe residing in Rajasthan.

PHARMACOLOGICAL PROPERTIES OF SELECTED ETHNOMEDINICAL PLANTS

1. *Gloriosa superba* L.

Ethnomedicinal usage: *G. superba* has been utilised for medicinal purposes across various cultures, including India, since ancient times. Different parts of the plant have been employed to treat a range of ailments (Trivedi, 2006). The powdered form of the tuber is utilised for treating skin diseases, chronic ulcers, and intestinal worms. It is also known to have the ability to induce labour-pain (Shahid *et. al.*, 2013; Samy *et. al.*, 2008; Pattanaik *et. al.*, 2008; Choudhary *et. al.*, 2008; Patil *et. al.*, 2008; Murty & Venkaiah, 2010; Kala *et. al.*, 2004; Lingaraju *et. al.*, 2013; Dwivedi *et. al.*, 2008; Jain & Singh 2010; Kirtikar, 2003; Boopathi, 2019; Senthilkumar, 2013; Clewer *et. al.*, 1915; Sharma *et. al.*, 2019; Srivastava *et. al.*, 2014; Gumustas *et. al.*, 2016; Dvorackova *et. al.*, 1984; Joshi *et. al.*, 2010; Kannan *et. al.*, 2007). The tuber paste is employed for the treatment of parasitic skin ailments (Trivedi, 2006). The tuber has been utilised for the treatment of snake bites and joint inflammation, as documented in (Badwaik *et. al.*, 2011; Padmapriya *et. al.*, 2015; Samy *et. al.*, 2015). The leaves of *G. superba* have been employed for the treatment of asthma, ulcers, haemorrhoids, and wound healing. The infusion of leaves is additionally utilised for the treatment of cough and pain (Schmelzer & Gurib-Fakim, 2008; Samy *et. al.*, 2008). Charaka documented the utilisation of the substance for the alleviation of labour pain, treatment of skin ailments, and as a means of promoting bowel movements. Sushruta documented the application of this substance for the treatment of obesity, ulcers, skin ailments, and as an antiseptic agent. The oil extract and paste derived from the tuberous root have been reported to possess therapeutic properties for skin ailments such as haemorrhoids, boils, and scrofula. Additionally, it is a constituent of the ethnomedicinal preparation in the Bhavaprakash, recommended for managing dermatological conditions (Khare, 2004).

i. *Antibacterial and Antifungal*

In various studies, extracts obtained from different parts of *G. superba* exhibited noteworthy efficacy against diverse bacteria and fungi. In an antimicrobial investigation, diverse solvent extracts of the seed and tuber components to evaluate their antibacterial efficacy. The findings revealed that the methanolic extract derived from the seed and tuber demonstrated the highest level of zone of inhibition. The antibacterial properties of leaf and stem extract were also evaluated. Also, it was observed that the extract obtained from tubers exhibited noteworthy antimicrobial activity against *S. aureus* and *E. coli* (Chimahali *et. al.*, 2019). These observations were reported in a study (Gopinath *et. al.*, 2014). Several studies have documented the antifungal properties of the rhizome and tuber components of *G. superba*. The N-butanol fractions extracted from the rhizome demonstrated noteworthy inhibitory effects against *C. albicans* and *C. glaberata* (Khan *et. al.*, 2008). According to a separate investigation, the tuber extract containing ethanol exhibited significant antifungal activity against *Mucor sp., A. niger* and *C. albicans* (Chimahali *et. al.*, 2019).

ii. *Anticancer activity*

Several anticancer studies have been conducted on *G. superba*, validating its potential efficacy against various types of carcinoma. The cytotoxic effect of various concentrations of methanolic tuber extract was assessed on a human hepatoma cell line in a research study. In study (Ghosh *et. al.*, 2016), the anticancer activity of silver and gold nanoparticles derived from tuber extract was also evaluated. The results indicated that the nanoparticles exhibited a more pronounced anticancer effect than platinum nanoparticles. This study was documented in (Rokade *et. al.*, 2016). A recent investigation has demonstrated that the methanol extract derived from the seed and its corresponding silver nanoparticle have displayed a noteworthy anticancer impact by increasing the lifespan of mice bearing tumours (Saradhadevi *et. al.*, 2017).

iii.Anti-Inflammatory Activity

Several anti-inflammatory studies were conducted on *G. superba*, and the findings indicated its significant potential in the treatment of inflammation. The anti-inflammatory activity of an extract obtained from the aerial parts was assessed. The hydroalcoholic extract of the aerial part displayed a significant inhibition of paw inflammation (John *et. al.*, 2009). In a separate investigation, the extract derived from tubers exhibited a significant anti-inflammatory impact during pharmacological screening, as reported by Mathur *et. al.* (2011). The results showed that the inflammation was significantly reduced by colchicine in comparison to other components (Joshi *et. al.*, 2010).

iv. Antidiabetic activity

The hypoglycemic potential of leaf extract was evaluated, wherein the extract derived from leaves exhibited a significant decrease in the level of blood glucose in animals used for experimentation. The analysed extracts demonstrated a hepatoprotective effect and a significant decrease in serum levels of cholesterol, triglycerides, lipid, and urea. Histopathological analysis revealed an increase in the size of beta-cells in the pancreas (Thakur *et. al.*, 2015). Furthermore, tubers exhibited significant hypoglycemic effects in an assessment of their antidiabetic properties (Rehan *et. al.*, 2010).

v. Anthelmintic activity

The anthelmintic activity of *G. superba* was evaluated by testing its ethanolic and aqueous extracts. A study was conducted wherein an ethanolic extract of tubers exhibited significant anthelmintic activity against *P. posthuma* (Suryavanshi *et. al.*, 2012).

vi. Hepatoprotective

The effects of this activity were analysed using an extract from the tuber of a plant. The findings demonstrated a notable improvement in the heightened levels of hepatic enzymes, including ALP, AST, ALT, ACP, and LDH (Mohandass & Indhumathi, 2011). Likewise, the aqueous extract derived from the tuber demonstrated a noteworthy hepatoprotective impact through the reduction of lipid peroxidation levels in hepatotoxicity induced by paracetamol (Indhumathi & Erattarakkal, 2011). The hepatoprotective effects of the leaf extract of *G. superba* were evaluated using the CCl_4-induced hepatotoxicity method. The results showed that the extract normalised the altered levels of hepatic enzymes (Samal, 2013).

vii.Analgesic

The pharmacological screening of the aerial parts showed its analgesic activity. The findings indicated that the hydroalcoholic extract elicited analgesic effects by prolonging the response time to pain stimuli in the subjects during the pharmacological screening (John *et. al.*, 2009).

viii.Antiulcer

The pharmacological screening was conducted to evaluate the antiulcer activity of the tuber. Empirical evidence indicates a significant decrease in ulceration resulting from the administration of aspirin in rats. During antiulcer screening, it was observed that extract reduced gastric secretion (Mna *et. al.*, 2013).

2. *Hygrophila auriculata* (Schumach.) Heine

Ethnomedicinal usage: The medicinal properties of *H. auriculata* have been extensively documented in historical medical literature, with various components of the plant being utilised to address a range of health conditions. The root component is recognised for its diuretic properties and is utilised in the management of urinary ailments such as kidney stones. The decoction is utilised for treating hepatic obstruction, rheumatism and gonorrhoea (Kapoor, 2018). The seeds have been utilised in the treatment of blood disorders, gastrointestinal ailments and fever. Additionally, they are incorporated as components in diverse aphrodisiac preparations. A mixture of seeds and buttermilk is administered as a remedy for diarrhoea. The leaves exhibit various therapeutic properties including diuretic, aphrodisiac and hypnotic effects. They are also employed in the treatment of gastrointestinal disorders, blood disorders, kidney stones and joint pain (Pattanaik *et. al.*, 2008). The leaves and seeds of certain plants are utilised for medicinal purposes in the treatment of jaundice. Charaka documented the utilisation of a decoction made from leaves in the treatment of kidney stones (Khare, 2004).

i. Aphrodisiac and Spermatogenic activity

A fraction enriched with alkaloids was derived from an extract of dried seed powder from *H. auriculata*. The fraction was subsequently evaluated for its potential aphrodisiac and spermatogenic properties. A study was conducted on rats to screen for aphrodisiac and spermatogenic activity. The study's findings indicate that the alkaloidal fraction had a significant impact on the serum testosterone level and testes weight, as well as an increase in libido during behavioural studies. The histological analysis suggests that the alkaloidal fraction has a notable spermatogenic potential, as reported in study (Vyas & Raval, 2015).

ii. Anti-inflammatory activity and antipyretic activity

The anti-inflammatory activity of petroleum ether, chloroform, and alcohol leaf extracts from *H. auriculata*. The findings indicate that the alcoholic and chloroform extracts led to a significant decrease in the inflammation. Antipyretic activity was also evaluated in pharmacological screening. The administration of chloroform and alcoholic extracts resulted in a significant reduction of elevated body temperature in comparison to the administration of aqueous and petroleum extract, as reported in (Patra *et. al.*, 2009).

iii.Antimicrobial activity and antifungal activity

Various studies have reported the antimicrobial properties of *H. auriculata*. The antimicrobial activity of a methanolic leaf extract was assessed on multiple bacterial strains (Samy, 2005). Furthermore, the efficacy of leaf extract was evaluated against Escherichia coli and Staphylococcus aureus. The results indicated that the chloroform and alcoholic extracts exhibited a significant zone of inhibition in comparison to the aqueous and petroleum extracts (Patra *et. al.*, 2008). Additionally, the alcoholic leaf extract exhibited noteworthy antifungal activity against *A. niger* in comparison to other fungi (Esther *et. al.*, 2012). The antimicrobial and antifungal activity of methanolic leaf extract, was reported in a study (Doss, 2013). The entire plant extract exhibited notable antimicrobial and antifungal properties as well (Hussain & Kumaresan, 2013). The antibacterial activity of Root extract was evaluated and demonstrated noteworthy inhibitory effects (Venugopalan & Nimisha, 2019).

iv. Anti-cancer activity

Studies have demonstrated that *H. auriculata* exhibits significant anti-cancer properties. A study was conducted wherein the petroleum-ether root extract was prepared and administered to mice. The results showed a significant reduction in tumour fluid volume and an increase in the lifespan of the test subjects, as documented in Mazumdar *et. al.* (1997). Seed methanolic extract exhibited significant anti-cancer properties and the formation of tumours, as reported in Ahmed *et. al.* (2001). In addition, the whole plant extract demonstrated a significant anticancer effect in comparison to animals in the control group (Pattanayak & Sunita, 2008). In a separate investigation, the efficacy of a whole plant extract was evaluated for its potential anticancer properties in rats. Experimental findings indicate that the administration of the extract resulted in a significant reduction in both tumour size and lipid peroxidation levels (Nair *et. al.*, 2015).

v. Antidiabetic activity

The anti-diabetic potential of the aqueous extract of the entire plant was evaluated using fasting blood glucose level and glucose tolerance test. The findings indicate that the aqueous extract effectively decreased the fasting glucose level and improved glucose tolerance in the experimental animals (Fernando *et. al.*, 1989). The hydroalcoholic extract derived from the aerial part demonstrated a significant hypoglycemic effect by reducing the level of blood glucose (Vijaykumar *et. al.*, 2006). Various seed extracts, including aqueous, methanol, ethanol, and chloroform, were evaluated for their antidiabetic properties. The findings revealed that the methanolic extract exhibited the most significant inhibition compared to the other extracts (Rastogi *et. al.*, 2014). Furthermore, the methanolic extract obtained from the aerial part of the plant exhibited a significant dose-dependent decrease in blood glucose levels in rats (Thorve *et. al.*, 2012).

MATERIALS AND METHODS

i. Collection and authentication of plant material

The tubers of *Gloriosa superba* and leaves of *Hygrophila auriculata* were picked and gathered in August from the forest areas of Rajasthan. The plant materials were subjected to a process of washing with water and subsequent drying under shade. The dried plant materials were then subjected to coarse grinding for further analysis. The taxonomic classification of these species was verified using the voucher specimens present in the KEW virtual herbarium.

ii. Extraction

The defatting of dried materials was carried out using petroleum ether and the Soxhlet apparatus for a duration of 30 hours until the supernatant in the Soxhlet achieved transparency. Subsequently, aqueous and ethanolic extracts of specific plant matter were procured utilising distilled water and ethanol, correspondingly (Petlevski *et. al.*, 2013; Kisseih *et. al.*, 2015). The extracts were subjected to filtration, followed by removal of the solvent using a lyophilizer and rotatory evaporator, resulting in the production of dried extracts (Espinoza *et. al.*, 2019). Percent yield of ethanolic and aqueous extracts obtained from the tuber of Gloriosa superba and the leaf of Hygrophila auriculata were determined by (Nfambi *et. al.*, 2015):

$$Percentage\ yield = \frac{Weight\ of\ final\ dry\ extract}{Weight\ of\ soaked\ plant\ extract} \times 100$$

iii.*In-vitro* pharmacological screening for efficacy of extracts

The experimental materials necessary for this study include ELISA Kits, MTT, DMS, dexamethasone, Griess reagent, PBS and LPS.

a. Nitrite Quantification Assay

Nitric oxide (NO) is an established indicator of the immunological function of macrophages (Paradkar *et. al.*, 2017). Macrophage suspensions in their raw state were introduced into a single well of a microtitre plate and subjected to a 24-hour incubation period within a CO2 incubator. Following a period of 24 hours, the aged media is substituted with newly prepared media. Macrophages were pre-treated with varying concentrations of nontoxic extract, phytochemicals, and standard drug. Subsequently, lipopolysaccharide (LPS) was introduced into every well, except for the control, and left to incubate for an additional 24 hours. Following the conclusion of the 24-hour incubation period with the experimental compound, the supernatants from each well are harvested and combined with a reagent. The resulting mixture is then incubated in a light-free environment for a brief duration. Subsequently, the level of light absorption was measured for every supernatant sample at a wavelength of 540 nm, and the concentration of nitric oxide was determined using a standard curve as previously described (Tan *et. al.*, 2015).

b. MTT assay

The MTT assay was conducted to ascertain the non-toxic concentrations of extracts and phytochemicals that exhibit the highest percentage of viability of macrophages. The cellular mixture was subjected to a 24 hr. incubation process in triplicate. Various concentrations of extracts and phytochemicals were introduced to macrophages that were seeded in a well, followed by incubation for a period of 24 hr. with extracts and phytochemicals, PBS was introduced and subsequently incubated for 3 hours. Subsequently, the substance was gathered and DMSO was introduced. Subsequently, the measurement of the degree to which a substance absorbed light was taken at a wavelength of 570 nm. The percentage of viability was determined for every chosen concentration of extract and phytochemicals by utilizing the formula (Tan *et. al.*, 2015; Mahendran *et. al.*, 2021).

Results and Discussion

In-vitro screening for immunomodulatory activity of extracts

The MTT assay was utilized to determine non-toxic concentrations of a chosen plant extract for in-vitro screening of immunomodulatory properties. A macrophage cell line was subjected to the assay. The assay was conducted on *G. superba* extract, resulting in a viability of over 90%. Various concentrations were then chosen for subsequent in-vitro immunomodulatory screening. On the contrary, the viability percentage of *H. auriculata* extract concentrations was found to be good. The concentrations of 10, 25, and 50 μg/ml were chosen based on their maximum viability percentage. Various concentrations of Rutin exhibited over 90% viability, prompting the selection of distinct concentrations for subsequent in-vitro investigations.

Effects of extracts on NO production

Nitric oxide is a signalling molecule that is produced during an immune response and plays a regulatory role in the functional activity of various immune cells [22]. The current study aimed to examine the impact of plant extracts on the production of NO in macrophages. The findings revealed that certain plant extracts and phytochemicals have a noteworthy effect on the production of NO. The findings indicate that the macrophages that were treated exhibited a substantial increase in the release of NO, which was comparable to the response observed with the standard drug. Significant inhibition on nitric oxide production was observed with higher doses of colchicine. On the contrary, the concentrations of 25 and 50 μg/ml exhibited a more noteworthy decrease in NO levels, which is analogous to the response of the standard drug. It was discovered that rutin exhibited a moderate inhibitory impact on the production of nitric oxide. The results indicate that the ethanolic extract of *H. auriculata* had a greater impact on the production of NO than rutin.

The findings suggest that the chosen plant extracts contain valuable bioactive compounds that may be accountable for their ability to inhibit the production of nitric oxide in macrophages. Previous studies have reported that phytochemicals such as alkaloids, flavonoids, terpenoids, and steroids have significant inhibitory potential against the production of NO. This finding is consistent with the results of the NO level assay conducted in the present research.

CONCLUSION

The current study represents a preliminary effort in identifying naturally occurring immunomodulatory agents that may be utilised in the management of immune-mediated disorders. The Indian state of Rajasthan is characterised by a diverse range of plant species, many of which possess significant ethnomedicinal properties. In the current study, *Gloriosa superba* and *Hygrophila auriculata* were chosen based on their traditional use in indigenous medicine and evaluated for their ability to modulate the immune system. In this scientific study, various in-vitro and in-vivo pharmacological screening techniques were employed, to elucidate the immunomodulatory properties of the substance under investigation and the underlying mechanisms responsible for these effects.

REFERENCES

1. Ade, R., & Rai, M.K. 2009. Current Advances in *Gloriosa superba* L. Biodiversitas Journal of Biological Diversity, 10(4).
2. Ahmed S, Rahman A, Mathur M, Athar M, Sultana S. 2001. Anti-tumor promoting activity of Asteracantha longifolia against experimental hepatocarcinogenesis in rats. Food and Chemical Toxicology. 39(1):19-28.

3. Ali, NAA, Juelich, WD, Kusnick, C and Lindequist U. 2001. Screening of Yemni medicinal plants for antibacterial and cytotoxic activities. Journal of Ethnopharmacology, 74: 173-179.
4. Amalraj, A.V., 1982. Promising plant resources for Indian Desert. J. Econ. Tax. Bot., 3(2): 349-354.
5. Badwaik, H., Giri, T. K., Tripathi, D. K., Singh, M., & Khan, A. H. 2011. A Review on Pharmacological Profile for Phytomedicine Known as Gloriosa superba Linn. Research Journal of Pharmacognosy and Phytochemistry, 2011, 3, 5.
6. Bhandari, M.M., 1974. Famine Foods of Rajasthan Desert. Econ. Bot., 28: 73-81.
7. Boopathi CA. 2019. Medicinal & Poisonous Plants of India. MJP Publisher.
8. Chimahali JO, Jebamalar AN, Duraikannu GA, Thirumal SI. 2019. Phytochemical analysis and evaluation of antimicrobial activity in the whole plant extracts of Gloriosa superba. Phytochemical Analysis. 12(6):245-9.
9. Chopra, I.C., B.K. Abrol and K.L. Handa, 1960. Medicinal Plants of Arid Zones. Part-I. Res. Ser., 13: 11-53.
10. Choudhary, K., Singh, M., & Pillai, U. 2008. Ethnobotanical survey of Rajasthan-An update. American-Eurasian Journal of Botany, 1(2):38-45.
11. Clewer, H. W. B., Green, S. J., & Tutin, F. XCI. 1915. The constituents of Gloriosa superba. Journal of the Chemical Society, Transactions, 107:835-846.
12. Cordell, GA. 1993. Pharmacognosy. New roots for an old science. In: A. Rahman, and F.Z. Basha (eds.) Studies in natural products chemistry, Vol. 13 Bioactive natural products (Part A). Elsevier.
13. Dixit, R.D. and R. Mishra, 1976. Studies of ethnobotany-II on some less known medicinal plants of Ajmer forest division, Rajasthan. Nagarjun, 19: 20-22.
14. Doss A, An SP. 2013. Antimicrobial activity of Hygrophila auriculata (Schumach.) Heine and Pergularia daemia Linn. African Journal of Plant Science. 7(4):137- 42.
15. Dvoráčková, S., Sedmera, P., Potěšilová, H., Šantavý, F., & Šimánek, V. 1984. Alkaloids of Gloriosa superba L. Collection of Czechoslovak chemical communications. 49(6): 1536-1542.
16. Dwivedi, S., Dwivedi, A., & Dwivedi, S. N. 2008. Folklore uses of some plants by the tribes of Madhya Pradesh with special reference to their conservation. Ethnobotanical leaflets. (1): 105.
17. Edeoga, HO, Okwu, DE and Mbaebie, BO. 2005. Phytochemical constituents of some Nigerian Medicinal plants. African Journal of Biotechnology, 4: 685-688.
18. Elizabeth M., David T.O. and Fred J.E. 1978. Phamnacological methods. Phytother Res. 1:1-7.
19. Espinoza J, Urzúa A, Sanhueza L, Walter M, Fincheira P, Muñoz P, Mendoza L, Wilkens M. 2019. Essential oil, extracts, and sesquiterpenes obtained from the heartwood of Pilgerodendron uviferum act as potential inhibitors of the Staphylococcus aureus NorA multidrug efflux pump. Frontiers in Microbiology. 10:337.
20. Esther VC, Saraswathi R, Dhanasekar S. 2012. In vitro antibacterial and antifungal activities along with x-ray irradiation studies of medicinal plant. Hygrophila auriculata. Int. J. Pharm. Pharm. Sci. 4:352-8.
21. Fernando MR, Wickramasinghe SN, Thabrew MI, Karunanayaka EH. 1989. A preliminary investigation of the possible hypoglycaemic activity of *Asteracanthus longifolia*. Journal of ethnopharmacology. 27(1-2):7-14.
22. Ghosh, S., Harke, A. N., Chacko, M. J., Gurav, S. P., Joshi, K. A., Dhepe, A., & Banerjee, K. 2016. *Gloriosa superba* mediated synthesis of silver and gold nanoparticles for anticancer applications. Journal of Nanomedicine & Nanotechnology. 7(390): 2.
23. Gopinath, K., Karthika, V., Gowri, S., Senthilkumar, V., Kumaresan, S., & Arumugam, A. 2014. Antibacterial activity of ruthenium nanoparticles synthesized using Gloriosa superba L. leaf extract. Journal of Nanostructure in Chemistry. 4(1): 83.
24. Gumustas, M., Polat, D. Ç., Kılıç, C. S., Akalın, K., Ozkan, S. A., & Coşkun, M. 2016. Comparison of Seeds of *Colchicum speciosum* and *Gloriosa superba* in respect to Colchicine and Colchicoside Contents by RP-LC. Natural product communications. 11(3): 397-400.
25. Gupta, R.K and B.K. Dutta, 1967. Vernacular names of the useful plants North-West Indian arid regions. J. Agri. Trop. Bot. Appl., 14: 402-452.
26. Gupta, R.K. and S.K. Saxena. 1968. Resources Survey of *Salvadora olecides* and *S. persica* as oil yielding plants in arid regions of India. Trop. Ecol., 8(2): 140-152.
27. Gupta, R.K., 1970. Resources survey of Gummiferous *Acacias* in Western Rajasthan. Trop. Ecol., 10(2): 140-161.
28. Harshberger, J.W., 1896. The purpose of ethnobotany. Bot. Gaz., 21: 146-158.
29. Hussain AZ, Kumaresan S. 2013. GC-MS analysis and antimicrobial activity of Hygrophila auriculata. Archives of Applied Science Research. 5(5):163-8.
30. Indhumathi T and erattarakkal S. 2011. Antioxidant activity of Gloriosa superba against paracetamol induced toxicity in experimental rats. IJPSR. 2(12): 212-215
31. Jain, S. P., & Singh, J. 2010. Traditional medicinal practices among the tribal people of Raigarh (Chhatisgarh), India. Indian Journal of Natural Products and Resources. 1(1): 109-115.
32. John JC, Fernandes J, Nandgude T, Niphade SR, Savla A, Deshmukh PT. 2009. Analgesic and anti-inflammatory activities of the hydroalcoholic extract from *Gloriosa superba* Linn. IJGP. 3(3).

33. Joshi, C. S., Priya, E. S., & Mathela, C.S. 2010. Isolation and anti-inflammatory activity of colchicinoids from *Gloriosa superba* seeds. Pharmaceutical biology. 48(2): 206-209.
34. Joshi, P., 1982. An Ethnobotanical study of Bhils: A preliminary study. J. Econ. Tax. Bot., 3: 257-266.
35. Kala, C.P., Farooquee, N.A., & Dhar, U. 2004. Prioritization of medicinal plants on the basis of available knowledge, existing practices and use value status in Uttaranchal, India. Biodiversity & Conservation. 13(2): 453-469.
36. Kannan, S., Wesley, S. D., Ruba, A., Rajalakshmi, A. R., & Kumaragurubaran, K. 2007. Optimization of solvents for effective isolation of colchicines from Gloriosa superba L. seeds. Natural product research. 21(5): 469-472.
37. Kapoor LD. 2018. CRC handbook of Ayurvedic medicinal plants. CRC press.
38. Khan, H., Ali Khan, M., Mahmood, T., & Choudhary, M.I. 2008. Antimicrobial activities of *Gloriosa superba* Linn (Colchicaceae) extracts. Journal of enzyme inhibition and medicinal chemistry. 23(6): 855-859.
39. Khare, C.P. 2004. Indian herbal remedies: rational Western therapy, ayurvedic, and other traditional usage, Botany. Springer science & business media.
40. King, G., 1869. Notes on famine foods of Marwar. Proc. Asiat. Soc. Bengal: 116-121.
41. King, G., 1870. Notes on vegetable products used as food during late famine in Rajputana. Trans. Bot. Soc. Edinb., 10: 198.
42. Kirtikar KR. 2003. The poisonous plants of Bombay. Scientific Publishers.
43. Kisseih E, Lechtenberg M, Petereit F, Sendker J, Zacharski D, Brandt S, Agyare C, Hensel A. 2015. Phytochemical characterization and in vitro wound healing activity of leaf extracts from Combretum mucronatum Schum. & Thonn.: Oligomeric procyanidins as strong inductors of cellular differentiation. Journal of ethnopharmacology. 174:628-36.
44. Lingaraju, D. P., Sudarshana, M. S., & Rajashekar, N. 2013. Ethnopharmacological survey of traditional medicinal plants in tribal areas of Kodagu district, Karnataka, India. Journal of pharmacy research. 6(2): 284-297.
45. Mahendran D, Kavi Kishor PB, Geetha N, Manish T, Sahi SV, Venkatachalam P. 2021. Efficient antibacterial/biofilm, anti-cancer and photocatalytic potential of titanium dioxide nanocatalysts green synthesised using Gloriosa superba rhizome extract. Journal of Experimental Nanoscience. 16(1):11-30.
46. Martin, A.J.S., 1983. Medicinal plant in Central, Chile. Econ. Bot., 37(2): 216-217.
47. Mathur, A., Verma, S. K., Singh, S. K., Mathur, D., Prasad, G. B. K. S., & Dua, V. K. 2011. Investigation of anti-inflammatory properties of *Swertia chirayta* and *Gloriosa superba*. Recent Research in Science and technology. 3(3).
48. Mazumdar UK, Gupta M, Maiti S, Mukherjee D. 1997. Antitumor activity of *Hygrophila spinosa* on Ehrlich ascites carcinoma and sarcoma-180 induced mice. Indian Journal of Experimental Biology. 35(5):473-7.
49. Merrill Singer, Pamela I. Erickson. 2011. A companion to medical anthropology. A John Wiley and sons' publication.
50. Mna, R., Chennamaneni, S. R., & Challa, S.R. 2013. Anti-ulcerogenic Evaluation of Gloriosa Superba Tuber Extracts In-vivo and Anthelmintic Activity In-vitro: A Comparison. Research and Reviews: Journal of Medical and Health Sciences. 2(4): 73-79.
51. Mohandass, S., & Indhumathi, T. 2011. Hepatoprotective efficacy of *Gloriosa superba* against paracetamol treated experimental rats-An in vivo study. Golden Research Thoughts. 1(3): 1-4.
52. Moteriya, P., Ram, J., Rathod, T., & Chanda, S. 2014. *In vitro* antioxidant and antibacterial potential of leaf and stem of *Gloriosa superba* L. American Journal of Phytomedicine and Clinical Therapeutics. 2(6): 703-18.
53. Murty, P.P., & Venkaiah, M. 2010. Some abortifacient plants used by the tribal people of Andhra Pradesh, India. Journal of Phytology. 2(4): 7-12.
54. Naika, H.R., Lingaraju, K., Manjunath, K., Kumar, D., Nagaraju, G., Suresh, D., & Nagabhushana, H. 2015. Green synthesis of CuO nanoparticles using *Gloriosa superba* L. extract and their antibacterial activity. Journal of Taibah University for Science. 9(1): 7-12.
55. Nair DV, Shridhar NB, Jayakumar K. 2015. Evaluation of anticancer activity of *Asteracantha longifolia* in 7,12-Dimethylbenz (a) anthracene-induced mammary gland carcinogenesis in Sprague Dawley rats. International Journal of Nutrition, Pharmacology, Neurological Diseases. 5(1):28.
56. Nair, R, Kalariya, T and Chanda S. 2005. Antibacterial activity of some selected Indian medicinal flora. Turk. J. Biol. 29: 41-47.
57. Nathawat, G.S. and B.D. Deshpande, 1960. Plants of economic importance from Rajasthan. Proc. Rajasthan Acad. Sci., 7: 38-47.
58. Nfambi J, Bbosa GS, Sembajwe LF, Gakunga J, Kasolo JN. 2015. Immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in Wistar albino rats. Journal of basic and clinical physiology and pharmacology. 26(6):603-11.
59. Padmapriya, S., Rajamani, K., & Sathiyamurthy, V.A. 2015. Glory lily (*Gloriosa superba* L.)-A review. International Journal of Current Pharmaceutical Review and Research. 7: 43-49.
60. Paradkar PH, Mishra LS, Joshi JV, Dandekar SP, Vaidya RA, Vaidya AB. 2017. *In vitro* macrophage activation: A technique for screening anti-inflammatory, immunomodulatory and anticancer activity of phytomolecules. 133-141.
61. Paroda, R.S., 1979. Plant resources of Indian arid Zone for Industrial Uses. In Arid Land Plant Resources, Eds. Goodin J.R. and D.K. Northinfton, Texas Tech. University: 261-281.

62. Pascual, ME, Carretero, ME, Slowing, KV and Villar, A. 2002. Simplified screening by TLC of plant drugs. Pharmaceutical Biology, 40(2): 139-143.
63. Patil, G.G., Mali, P.Y., & Bhadane, V.V. 2008. Folk remedies used against respiratory disorders in Jalgaon district, Maharashtra. Natural product radiance. 7(4): 354-358.
64. Patra A, Jha S, Murthy P. 2009. Phytochemical and pharmacological potential of *Hygrophila spinosa* T. anders. Pharmacognosy Reviews. 3(6): 330.
65. Pattanaik, C., Reddy, C. S., & Murthy, M.S.R. 2008. An ethnobotanical survey of medicinal plants used by the Didayi tribe of Malkangiri district of Orissa, India. Fitoterapia. 79(1): 67-71.
66. Pattanayak SP and Sunita P. 2008. Antitumor potency and toxicology of an Indian Ayurvedic plant, *Hygrophila spinosa*. Pharmacologyonline. 2(1):361-71.
67. Pawar, B.M., Wavhal, V.P., Pawar, N.D., Agarwal, M.R., Shinde, P.B., & Kamble, H.V. 2010. Anthelmintic activity of Gloriosa superba Linn (Liliaceae). International Journal of PharmTech Research. 2(2): 1483-1487.
68. Peters, C., I.L. Gentry, R.O. Schneider, G. Platais, D. Rosenblatt and Mendelsohn, 1989. Valuation of an Amazonian rainforest. Nature, 29: 655.
69. Petlevski R, Flajs D, Kalođera Z, Končić MZ. 2013. Composition and antioxidant activity of aqueous and ethanolic Pelargonium radula extracts. South African Journal of Botany. 85:17-22.
70. Raheja, P.C. and A.K. Sen, 1964. Resources in prospective. In Recent Development in Rajasthan, Central Arid Zone Research Institute, Jodhpur: 141-146.
71. Rastogi A, Shankar SR, Mahalingam GA. 2014. Phytochemical screening, antioxidant activity and in vitro anti-diabetic activity of aqueous, methanolic, ethanolic and chloroformic extracts of Hygrophila auriculata. Int J Pharm Pharm Sci. 6(5):557-60.
72. Rehan, A., Srivastava, S. P., Rakesh, M., Rajendran, S. M., Arya, K. R., & Srivastava, A. K. 2010. Antihyperglycaemic activity of certain medicinal plants. National Journal of Life Sciences, 7(1): 17-20.
73. Rokade, S. S., Joshi, K. A., Mahajan, K., Patil, S., Tomar, G., Dubal, D.S. & Ghosh, S. 2018. *Gloriosa superba* Mediated Synthesis of Platinum and Palladium Nanoparticles for Induction of Apoptosis in Breast Cancer. Bioinorganic chemistry and applications.
74. Samal, P.K. 2013. Hepatoprotective Activity of Gloriosa superba on Liver Damage Caused by CCL4 in Rats. Asian Journal of Pharmaceutical Research. 3(4): 194-197.
75. Samy RP, Thwin MM, Gopalakrishnakone P, Ignacimuthu S. 2008. Ethnobotanical survey of folk plants for the treatment of snakebites in Southern part of Tamilnadu, India. Journal of ethnopharmacology. 115(2): 302-12.
76. Saradhadevi, M., Gnanadesigan, M., Kapildev, G., & Vasanth, D. 2017. Dataset on antitumor properties of silver nanoparticles from *Gloriosa superba* (L.) seed on Dalton Lymphoma Ascites (DLA) tumor: Facile and biocompatible approach. Data in brief, 14, 524-530.
77. Sarvananda L & Premarathna AD. 2018. Ethnopharmacolological potential and medicinal uses of Hygrophila auriculata. Journal of Ayurvedic and Herbal Medicine. 4(4): 185-8.
78. Schmelzer, G. H., & Gurib-Fakim, A. 2008. Plant Resources of Tropical Africa 11 (1). Medicinal Plants 1. PROTA Foundation, Wageningen, Netherlands.
79. Schultes, R.E., 1992. Ethnobotany and technology in the Northwest Amazon: A partnership. In Sustainable harvest and marketing of rain forest products, Eds. Plotkin and Famolare, Island Press, CA: 45-76.
80. Senthilkumar, M. 2013. Phytochemical screening and antibacterial activity of *Gloriosa superba* Linn. International Journal of Pharmacognosy and Phytochemical Research, 5(1): 31-36.
81. Shahid M, Shahzad A, Malik A, Sahai A. 2013. Recent trends in biotechnology and therapeutic applications of medicinal plants. New Delhi: Springer.
82. Sharma, S., Sharma, Y.P., & Bhardwaj. 2019. Development and Validation of HPLC Method for the Determination and Quantification of Colchicine in *Gloriosa superba*. International Research Journal of Pure and Applied Chemistry: 1-9.
83. Simon S.E. & Jayakumar, F.A. 2016. Antioxidant activity and Anticancer study on phytochemicals extract from tubers of *Gloriosa superba* against human cancer cell (HEP-G2). Research and Reviews: Journal of Pharmacognosy and Phytochemistry: 7-12.
84. Singh, V. and B.B. Shetty, 1977. A Survey of natural plant resources in Rajasthan Desert. Trans. Isdt. Ucds. 2(2): 296-305.
85. Singh, V. and R.P. Pandey. 1982. Fiber yielding plants of Rajasthan. J. Econ. Tax. Bot., 3: 385-390.
86. Srivastava, S., Misra, A., Shukla, P. K., Kumar, B., Lata, S., & Rawat, A. K. S. 2014. A validated over pressured layered chromatography (OPLC) method for separation and quantification of colchicine in *Gloriosa superba* (L.) tubers from different geographical regions. RSC Advances. 4(105): 60902-60906.
87. Srivastava, T.N. 1977. Forest resources of Rajasthan. Natural Resources of Rajasthan, 1: 151-164.
88. Sudarshan SR. 1985. Encyclopaedia of Indian Medicine. Popular Prakashan.

89. Suryavanshi, S., Rai, G., & Malviya, S.N. 2012. Evaluation of anti-microbial and anthelmintic activity of *Gloriosa superba* tubers. Advance Research in Pharmaceuticals and Biologicals, 2(1): 45-52.
90. Tan WS, Arulselvan P, Karthivashan G, Fakurazi S. 2015. *Moringa oleifera* flower extract suppresses the activation of inflammatory mediators in lipopolysaccharide-stimulated RAW 264.7 macrophages via NF-κB pathway. Mediators of inflammation.
91. Thakur, V., Choudhary, M., Garg, A., Choudhary, N., Jangra, A. and Budhwar, V. 2015. Evaluation of a Hydroalcoholic Extract of the Leaves from the Endangered Medicinal Plant gloriosa superba linn. colchicaceae) for its Potential Antidiabetic Effect. Archives of Medicine. 7:6-8.
92. Thorve VS, Kshirsagar AD, Vyawahare NS, Thakurdesai PA, Bhandare AM. 2012. *H. Spinosa* T. Anders ameliorates diabetic neuropathy in wistar albino rats. Journal of Complementary and Integrative Medicine. 9(1):1-7.
93. Trivedi, P.C. 2006. Medicinal plants: traditional knowledge. IK International Pvt Ltd.
94. Vedavathy S. 2003. Scope and importance of traditional medicines. Indian Journal of Traditional Knowledge, 2(3): 236-23.
95. Venugopalan P and Nimisha CN. 2019. Antioxidant and antibacterial activity of Hygrophila spinosa T Anders root extracts. Asian Journal of Pharmacy and Pharmacology. 5(5):959-63.
96. Vijayakumar M, Govindarajan R, Rao GM, Rao CV, Shirwaikar A, Mehrotra S, Pushpangadan P. 2006. Action of *Hygrophila auriculata* against streptozotocin-induced oxidative stress. Journal of Ethnopharmacology. 104(3):356-61.
97. Vyas N, and Raval M. 2015. Quantification of two marker compounds from unsaponifiable matter of *Hygrophila spinosa* seeds using validated TLC-densitometric method. International Journal of Pharmaceutical Research.
98. Vyas, D.N. and R.S. Gupta, 1962. An annotated list of medicinal plants of Alwar, Rajasthan-I, Proc. Raj. Acad. Sci., 9(2): 49-52.
99. Zita T. 1997. Health care the herbal way. Eastern Pharmacist. 8:71.