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Research Article

Anti-proliferating activity of some toxic and medicinal plants used by Wancho tribe of Arunachal Pradesh, India

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Medicinal plants extend to show an imperative role in the rural healthcare system of developing countries, where herbal medicine has a continuous history of long use. Field studies were conducted following standard ethnobotanical techniques to collect information on the use of toxic and medicinal plants used by the Wancho tribe of Arunachal Pradesh. *Vigna radiata* was used as a plant model in this study to investigate the antiproliferative property of selected medicinal plant extracts. Green gram seedling root apical meristem cells were used to determine the inhibition of germination and slow growth. Different concentrations of plant extract (20, 50, 250 and 500 µg/ml) were treated in a test tube containing the green gram seeds (n=5) on time dependent manner. Colchicine (20 mg/ml) was used as a standard drug for the growth retardation of green gram seedlings, while the distilled water group served as negative control. Germination test of *Vigna radiata* L. was performed according to ISTA (International Rules for Seed Testing) rules. The present study concludes that the methanolic plant extracts of all the collected plants significantly inhibited the rate of seed germination and seedling growth at dose dependent manner. It signifies that the use of *Phlogacanthus parviflorus* and *Mikania micrantha* in high concentrations may be potentially therapeutic for inhibiting the cell cycle in eukaryotic organisms.

Keywords: anti-proliferative; Arunachal Pradesh; medicine; plants; toxic; Wancho

Introduction

Plants are not only an essential part of health care but also ensures safe medicines for the future (Hamburger and Hostettmann, 1991). Most of the relevant drugs which have transformed the modern medicinal practice have been isolated from plants. However, just 1-10% of all plant species on the planet have been chemically and pharmacologically investigated for their possible therapeutic benefits (Verpoorte, 2000). The chemical ingredients present in these medicinal herbal exhibit latent therapeutic properties. The plant-derived compounds have also been a major source of many clinically effective antiproliferative agents (Agyare *et al.*, 2013). The herbal extracts, which are an excellent scavenger of free radicals, have been used to treat a variety of diseases. It contains a variety of phytochemicals that work together to combat diseases that are resistant to purified compounds (Sharma, 2016). Flavopiridol, Epipodophyllotoxin, Noscapine, Paclitaxel,

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Vindesine, Vinorelbine, Vinflunine, Pomiferin, Roscovitine, Sulphoraphane, Vincristine, and Vinblastin are few examples of well-known plant derived anticancer drugs (Greenwell and Rahman, 2015; Pereira *et al.*, 2016, Basu and Maier, 2018).

The expansion and recognition of these medicinal herbs including the monetary aids of these plants are on the rise in both developed and developing nations (WHO, 1998). These herbs can be easily accessed by the common man and recognized to be much safer than synthetic drugs available in markets (Singh and Singh, 1981). Therefore, the screening of biologically active agents of these herbal plant extracts had led to the discovery of many pharmaceutically important drugs (Rastogi and Meharotra, 1996).

In the state of Arunachal Pradesh, the use of medicinal and toxic plants in the healthcare system and fishing respectively is an integral part of tribal culture. Twenty-eight major different ethnic groups are residing in diverse regions of the state and they have distinct traditional knowledge on the use of plants (Tag and Das, 2004). Their perception and knowledge of using these assets, however, varied from tribe to tribe. With the use of these plants on daily basis, their belief in their native plants for medications or fishing has evolved. These toxic and medicinal plants used by the indigenous people may also have anti-proliferating property. Therefore, this study was an attempt to collect the information available on the medicinal and toxic plants used by the Wanchos of Longding District, Arunachal Pradesh and to evaluate their anti-proliferative activity.

Materials and Methods

Study area and ethnic community

The Longding district situated in the Patkai range is comprised of mountainous terrain flowed by streams and rivers cascading down from upper radiant elevation (Figure 1). The Patkai range constitutes the part of the Eastern Himalayan mountains. Due to the consistent wind, the region is cold and a blanket of mist often conceals the area. The Wanchos inhabiting the District are tribal, ethnically linked to the Konyak Naga of Nagaland. Their dialect belongs to the Tibeto-Burman ancestry. The majority of the population is agrarian. Oriah is the main festival, which is a celebration of the sowing season. The society is traditionally administered by a council of Wangham or Chieftains. Both males and females actively participate in agriculture and other activities. Tattooing plays a significant role in the tribe, which signifies the valour (award for hunting enemy's heads in the past) for men and the attainment of womanhood for women.

Field study and data collection

Field studies were conducted following the standard ethnobotanical techniques of Martin (1995). The ethnobotanical survey covers the Wancho inhabited villages. The plant-related data was collected through Participatory Rural Appraisal (PRA), based on personal interaction with the indigenous population and practical observation in the survey. Standard questionnaires were devised to obtain information on the uses of plants in fishing and in the treatment of various ailments. A total of 30 informants were interviewed to record their knowledge, skills, and practices on plant-based folk medicines and toxic plants. The group interview was conducted for a comparative statement. Identification of these medicinal plants was done by referring to the herbaria of Botanical Survey of India (BSI), Itanagar, and BSI (Kolkata) and also through consultation of taxonomic literature and Floras. The scientific names of the collected plant specimens were verified by visiting the "theplantlist.org" website. The voucher specimens were deposited to the Plant Systematic and Ethnobotany Lab of Rajiv Gandhi University, Rono Hills, Arunachal Pradesh.

Preparation of plant extract

The Medicinal plants were collected from the study site (Table 1) and the fresh plant parts were washed, shade-dried, and grounded to powder. Ten grams of powder were dissolved in 100 ml of methanol overnight, filtered, and kept at 0 °C for further use.

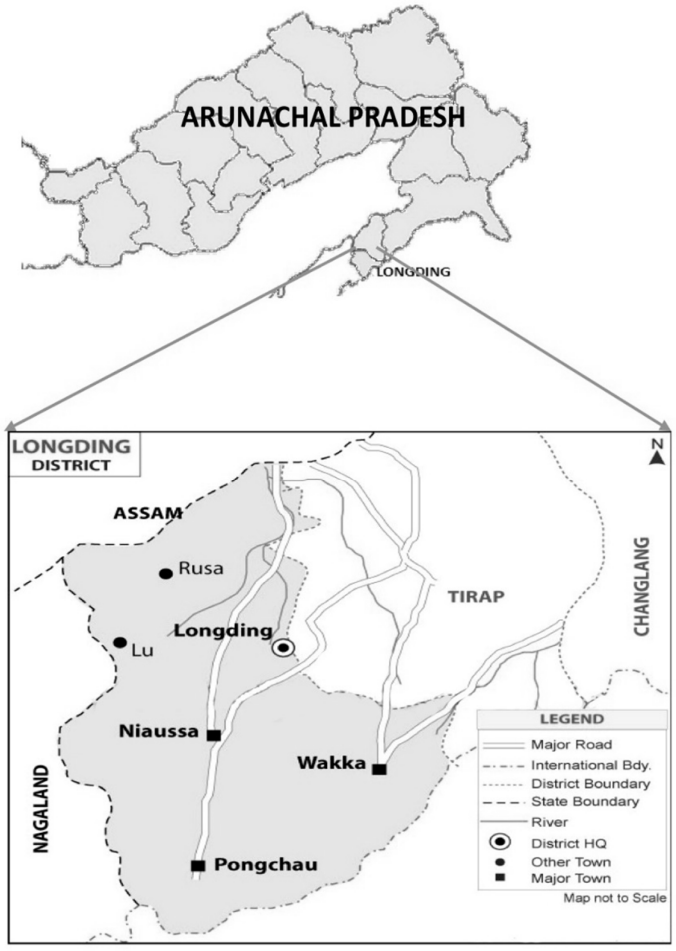

Figure 1. Map of Study area: Longding district of Arunachal Pradesh, India

Table 1. Enumeration of the medicinal and toxic plants used by Wancho community of Eastern Himalayas

Sl. No.	Botanical names	Family	Life forms	Vernacular names	Parts used	Mode of use	Traditional uses
1.	*Mikania micrantha*	Asteraceae	Creeper	Zusa	Wp	Paste	Wound healing, stomach disturbances
2.	*Ostodes paniculata*	Euphorbiaceae	Tree	Sojuh	Lv, Pt	Paste	Fish poisoning
3.	*Tithonia diversifolia*	Asteraceae	Shrub	Poshak	Lv	Decoction	Anti-inflammatory, anti-oxidant
4.	*Blumea balsamifera*	Asteraceae	Herb	Mailong	Lv	Decoction	Kidney diseases, common cold
5.	*Acorus calanus*	Acoraceae	Herb	Lannu	Wp	Decoction	Gastrointestinal disorders
6.	*Casearia zeylanica*	Salicaceae	Tree	Zookai	Lv, Pt	Paste	Fish poisoning
7.	*Phlogacanthus parviflorus*	Acanthaceae	Herb	Hingkhah	Lv	Decoction	Gastrointestinal disorders, chest pain

Note: Wp: whole plant, Lv: leaves, Pt: Petiole, Br: Bark

Experimental plant model

Good quality and diseased free *Vigna radiata* L. (green gram) were used as a model plant. Green gram seedling root apical meristem cells were used to determine the inhibition of germination and slow growth. Colchicine (20 mg/ml) is used as a standard drug for the growth retardation of green gram seedlings.

Treatment of Vigna radiata L. seedlings

Germination test of green gram was performed following standard rules (International Rules for Seed Testing, 2018). For the germination of seeds, different concentrations (20, 50, and 250 µg/ml) of plants extract treated with methanol were placed in a test tube, and the seeds of green gram (n=5) were added into each tube. The tubes were closed and the plant was allowed to germinate at room temperature. Colchicine (20 mg/ml) treated group served as a positive control, while the distilled water as negative control (Ray *et al.*, 2013). Seed germination and root length were measured after 72 hours both in control and test groups.

Results and Discussion

Taxonomic evaluation of the collected medicinal plants

A total of seven medicinal and toxic plants belonging to five plant families were collected and identified (Figure 2). Life forms consist of herbs (3 plants), trees (2 plants), shrubs (1 plant), and creepers (1 plant). Among the plant parts used, the leaves were most commonly used. The rest of the plant parts includes the petiole and the whole plant. The collected medicinal plants were found to be used in several ailments including wound healing, stomach disorders, anti-inflammatory, kidney diseases, common cold, gastrointestinal disorders, gastrointestinal disorders, and chest pain. While the toxic plants were actively used in traditional fish poisoning. The medicinal plants were administered through the preparation of decoction. Whereas, the paste of toxic plants was directly poured into the runner water while fishing.

Effects on seed germination and root length

This study evaluated the anti-proliferative potential of seven plants, prescribed mostly by traditional healers for various ailments. The methanolic plant extracts investigated in this study revealed dose inhibitory effects on seed germination and root length in *V. radiata*. A higher concentration of plant extracts (500 µg/ml) inhibited seed germination compared to the negative control of respective groups (Figure 3). 250 µg/ml of plant extracts treated group also inhibits the germination of green gram seeds at 72 hours of treatment when compared with the negative control (Figure 4). On the other hand, the colchicine (20 mg/ml) induced group showed the complete retardation of root growth in 24, 48, and 72 hours' time interval when compared to all the respective groups (Figure 4).

The lowest concentration (20 µg/ml) of plant extract of all plant species did not exhibit much effect on the germination and root length of green gram saplings. 50 µg/ml concentration, however, showed little effect and decreased the root length. 250 µg/ml concentration also exhibited a decrease in root length; while some plant (*Mikania micrantha* and *Phlogacanthus parviflorus)* extracts have shown complete retardation of root growth. The highest concentration (500 µg/ml) of methanolic extract of five plants exhibited high anti-proliferative potential. These plants were *P. parviflorus*, *C. zeylanica*, *M. micrantha*, *B. balsamifera and T. diversifolia*. On the contrary, the plant species *Ostodes paniculata* displayed ample growth of radicles in every dose. Thus, it is least effective than other competitors. According to Handa *et al.* (1983), the isolated compounds from the plant parts of *Ostodes paniculata* display significant *in vitro* cytotoxic effect on cancer cell lines. Considering this, it may be assumed that all other plants, showing better result than *O. paniculata* in current study may exhibit effective results with the cell line *in vitro*.

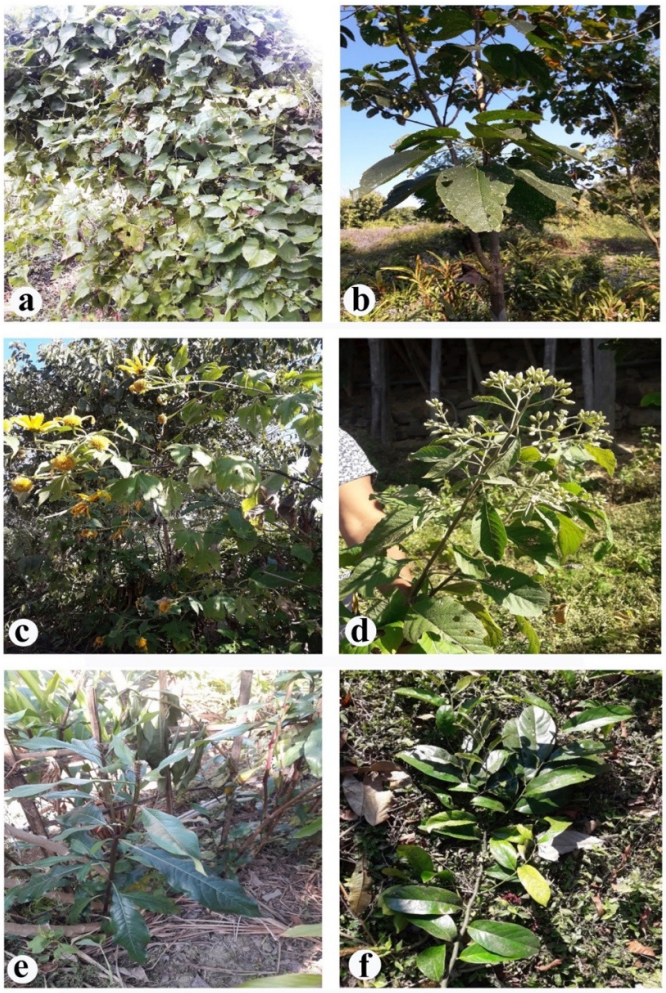

Figure 2. Some of the medicinal and toxic plants used by Wancho tribe of Arunachal Pradesh: (a) *Mikania micrantha*, (b) *Ostodes paniculata*, (c) *Tithonia diversifolia*, (d) *Blumea balsamifera*, (e) *Phlogacanthus parviflorus*, and (f) *Casearia zeylanica*

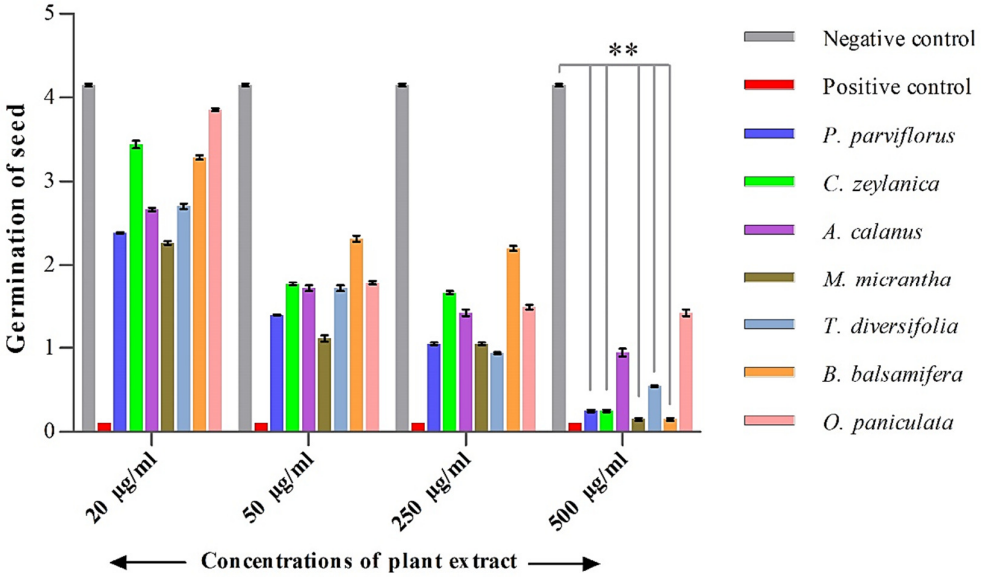

Figure 3. Effect of different methanolic plant extracts on the seed germination of *Vigna radiata*
Mean ± SEM (n=5). Two-way ANOVA followed by Bonferroni's post-test. Significance set at $P<0.05$. ¨$P<0.01$ compared to negative control.

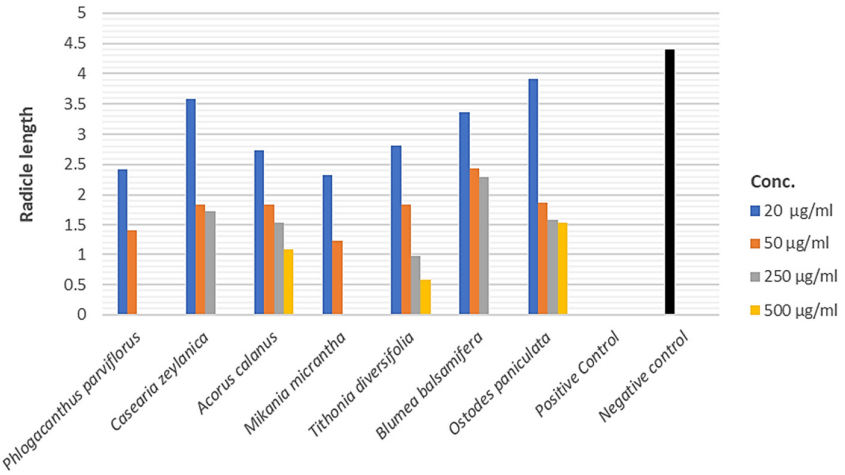

Figure 4. Clustered column displaying the effect of different methanolic plant extracts on the radicle length of *Vigna radiata* seeds

Several previous studies have reported the antiproliferative activity of different medicinal plant in *V. radiata* model (Charchafchi *et al.*, 2007; Fawzia *et al.*, 2007; Ray *et al.*, 2013; Chetry and Bahrali, 2018). It was also reported that the essential oil extracted from the invasive weed *M. micrantha* flower has been found to have antimicrobial and anticancer properties (Saikia *et al.*, 2020). The plant *M. mirantha* was also reported to possess anti-tumour activity (Dou *et al.,* 2014). On the other hand, the whole plant and crude extracts, as well as its isolated constituents of *B. balsamifera* display numerous biological activities, such as antitumor, hepatoprotective, superoxide radical scavenging, antioxidant, antimicrobial and anti-inflammation, anti-plasmodial, anti-tyrosinase, platelet aggregation, enhancing percutaneous penetration, wound healing, anti-obesity, along with disease and insect resistant activities (Pang *et al.*, 2014).

The anti-proliferative activity of the plant species *P. parviflorus* was not previously reported. However, other species of this genus (*P. thyrsiflorus*) were reported with the anti-cancerous along with other properties such as antibacterial, antifungal, anti-diabetic, anti-inflammatory, anti-cancerous, hypolipidaemic and hepatoprotective (Singh and Singh, 2010). It was reported that the leaves and flowers of *P. parviflorus* are traditionally cooked and consumed as vegetables; while, the decoction of the leaves helps in curing fever, cold and sore throat (Dutta and Nath 2019).

On the other hand, even though some workers have described the manifold uses of the medicinal or toxic plants, there is still a need of doing much work in this field (Perme *et al.*, 2015). Moreover, the present research was a preliminary investigation on the potential of locally available herbal toxic and medicinal plants extracts, which must be supported and validated further by performing the *in vivo* anti-proliferative activity was evaluating the tumour inhibitory rates in cell line or thymus index and spleen index of S180-bearing mice (Dou *et al.*, 2014).

Conclusions

The present study concludes that the methanolic plant extracts of all the collected plants significantly inhibited the rate of seed germination and seedling growth. It signifies that the use of *Phlogacanthus parviflorus* and *Mikania micrantha* in high concentrations may be potentially therapeutic for inhibiting the cell cycle in eukaryotic organisms. Thus, these two plants may contain strong anti-proliferative agents for the management of proliferative diseases such as cancer progression. However, further research will be needed to isolate the active compounds and to determine their influence on the cell cycle regulatory gene expression.

Authors' Contributions

TW: guided and drafted the manuscript. AW: performed the experiment. LBC: analysed data and edited the manuscript. ST: supervision. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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