A Review Paper on Medicinal Plants with Anti-Leukemic Effects

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ABSTRACT: ***Leukaemia has emerged as one of the most lethal of the cancers in the modern era. Out of all Leukaemia, Acute Myelogenous Leukaemia (AML) is the most notorious. The treatment regime being expensive & toxic has led to a world-wide hunt for alternate sources of anti-AML agents. In the various traditional systems of medicines, the Indian rose (Rosa indica) has been recorded as having various health benefits. In this paper, the Ethanolic extract of Rose was investigated for its anti-AML activity. The investigators treated both the AML cell line, HL-60 & the Normal Human Embryonic Kidney cell lines (NHEK) with varying doses of the Rosa indica's Ethanolic extract for 24 h. Based on the current observations, the Rosa indica's Ethanolic extract induced growth inhibition having*** IC_{50} ***44.44 µg/ml. Moreover, the ethanolic extract showed negligible activity against normal cell lines. Based on these findings, it can be safely assumed that the ethanolic fraction of the Indian Rose can be utilized as an anti-AML agent.***

KEYWORDS: ***Apoptosis, Acute Myelogenous Leukaemia (AML), Cells, Ethanolic Fraction,*** IC_{50} ***Value, HL-60, Reactive Oxygen Species (ROS), Rosa Indica.***

1. INTRODUCTION

Among adults, acute myelogenous leukaemia (AML) is a common form of leukaemia afflicting them. Though advances in modern medicine has led to an improvement in the life span of the younger population, yet people who are above the age of 65 bear the brunt of this disease. Though, AML can occur in patients with a pre-existing haematological disorder, or due to chemotherapy (involving radiation, alkylating agents, topoisomerases II etc.) though it appears many a times as de novo malignancy in erstwhile healthy people in many cases. It must be added that, the occurrence of AML entails the anomalous differentiation & proliferation of a doppelgänger populace of transformed stem cells of myeloid origin which occurs due to a cascade of various signalling molecules. WHO has classified AML according to some parameters which have been disclosed in Table 1 while Table 2 discloses the molecular & cytogenic profiling of risk groups[1].

Table 1: Classification of AML as per the WHO. Various sub-types of AML (AML) have been tabulated in this table based upon their chromosomal aberrations[1]**.**

Genetic anomalies	**Types**
AML having t(8:21)(q22;q22); RUNX1-RUNXITI	AML having periodic heritable abnormalities
AML having inv(16)(p13.1q22) or t(16;16)(p 13.1;q 22); CBFB-MYH11	
APL having PML-RARA	
AML having t(9;11)(p21.3;q23.3);BMLLT3-KMT2A	
ML having t(6,9)(p23;q34.1); DEK-NUP 214	
AML having inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM	
AML having minimal differentiation	AML having myelodysplasia-centered alterations
AML in absence of maturation	Thyroid centered myeloid neoplasms
AML in presence of maturation	

AML-M4	
AML-M5	
Category M6 leukaemia	
AML-M7	
Type M0 leukaemia	
Transient abnormal myelopoiesis	Myeloid sarcoma
ML in combination of trisomy 21	Combination of Myeloid proliferation and trisomy 21

Table 2 : Molecular & Cytogenic profiling of risk groups. In AML, the major factor for the phenomenon of overall survival (OS) & complete remission (CR) is cytogenetic changes[2]**.**

Molecular anomalies & cytogenetic profile	**Cytogenetic profile**	**Prognostic-risk factor**
T(8:21)(q22;q22) lacking c-KIT alteration Inv(16)(p13;1q22) t(15;17)(q22;q12)	T(8:21)(q22;q22) Inv(16)(p13;1q22) t(15;17)(q22;q12)	Favourable
Altered NPM1 in absence of FLT3-ITD(CN-AML) Altered biallelic CEPBA (CN-AML) T(8:21)(q22;q22) having altered c-KIT CN-AML except those involved within beneficial o0r meagre predictive assembly t(9;11)(p22;q23)	CN-AML t(9,11)(p22;q23) Cytogenetic aberrations excluded within complimentary or poor predective jeopardy aspects	Median
Cytogenetic anomalies excluded within beneficial or poor predictive menace sets TP53 alteration notwithstanding with cytogenetic outline CN having FLT3-ITD CN having DNMT3A CN having KMT2A-PTD Inv(3)(q21q26.2) t(6;9)(p23;q34) 11q anomalies apart from t(9;11) -5 or del (5q) -7 Intricate karyotype	Inv(3)(q21 q26.2) t(6;9)(p23;q34) 12q anomalies excluding t(9;11) -5 or del (5q) -7 Intricate karyotype	Adverse

1.1 Common Kinase Signalling Alterations:

The most important mutations in the kinase signalling pathway, involves the genes encoding the trans-membrane receptors of tyrosine kinase such as FLT3 & KIT, or the genes encoding the guanosine triphosphatases which is a member of the RAS family, (**Figure 1**). Upon DNA sequencing of clinical samples, such mutations have been observed in 30% to 75% of patients[3].

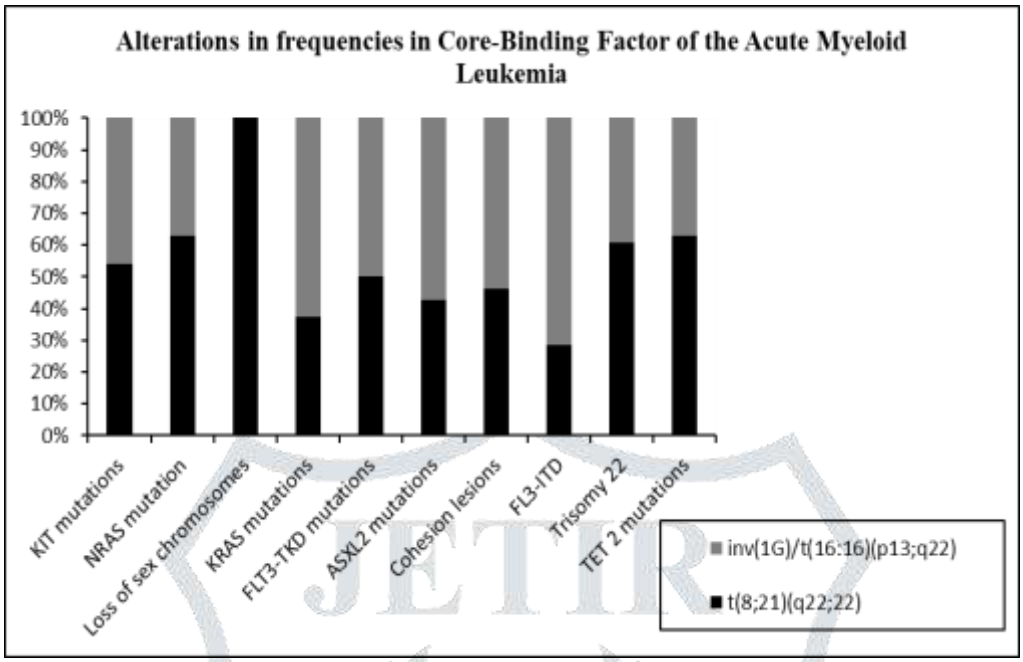

Figure 1: Mutation landscape analysis of clinical samples (A) Genetic landscape & (B) Alterations in genetic mutation frequencies in AML[3]**.**

1.2 Secondary AML:

AML occurring due to a previous myeloid malignancy, or the one that occurs after previous radiation therapy or chemotherapy for a separate malignancy are known as secondary or 2° AML. Secondary AML befalls upon senior citizens & studies based upon population have suggested that such AML comprises of nearly 25% AML cases, in which 17% to 25% evolves via preceding myeloid ailment & 6% to 10% because of remedy induced (Figure 2)[4].

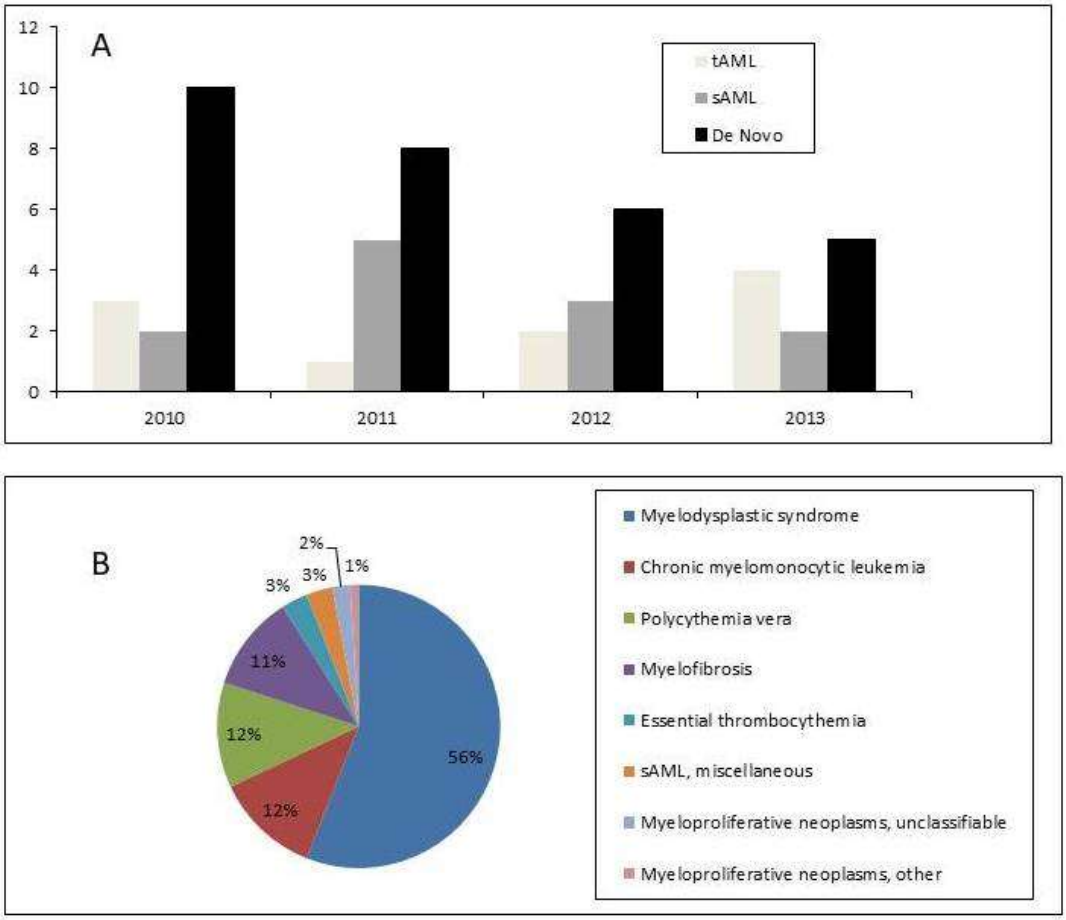

Figure 2. (A) Incidence of AML occurrence based upon their origin: Secondary, De Novo & cancer therapy induced (AML) in a Population of Denmark on- 3,000 non-selected Patients having AML, (B) Spread of previous blood borne Disease in 600 Patients possessing 2° AML[4]**.**

1.3 TP53 mutated AML:

In AML, mutations in TP53 gene are mainly found in the DNA-binding domain. Interestingly, among AML patients, TP53 gene mutations arise within about 11% to 18% of the AML instances. Among the genes that define AML, reduced frequencies of co-occurring mutations were reported in FLT3 & NPM1. Similar reduction in frequencies was reported in genes namely; WT1, DNMT3A, IDH1, RUNX1 & IDH2 along with a spurt in the frequency of JAK2 deviants. There is also a report about the TP53 mutation which reacted to decitabine, & a DNMT3A mutation was noted in a different clone which inflated during remission & was then removed at relapse (Figure 3A). In an earlier report, about 65 obtainable cases with simultaneous mutations in DNMT3A, N/KRAS FLT3 or TET2 (which are one of the most co-mutated common genes) wherein, TP53 deviant allele prevalance & simultaneous alteration were publically obtainable were determined (Figure 3B)[5].

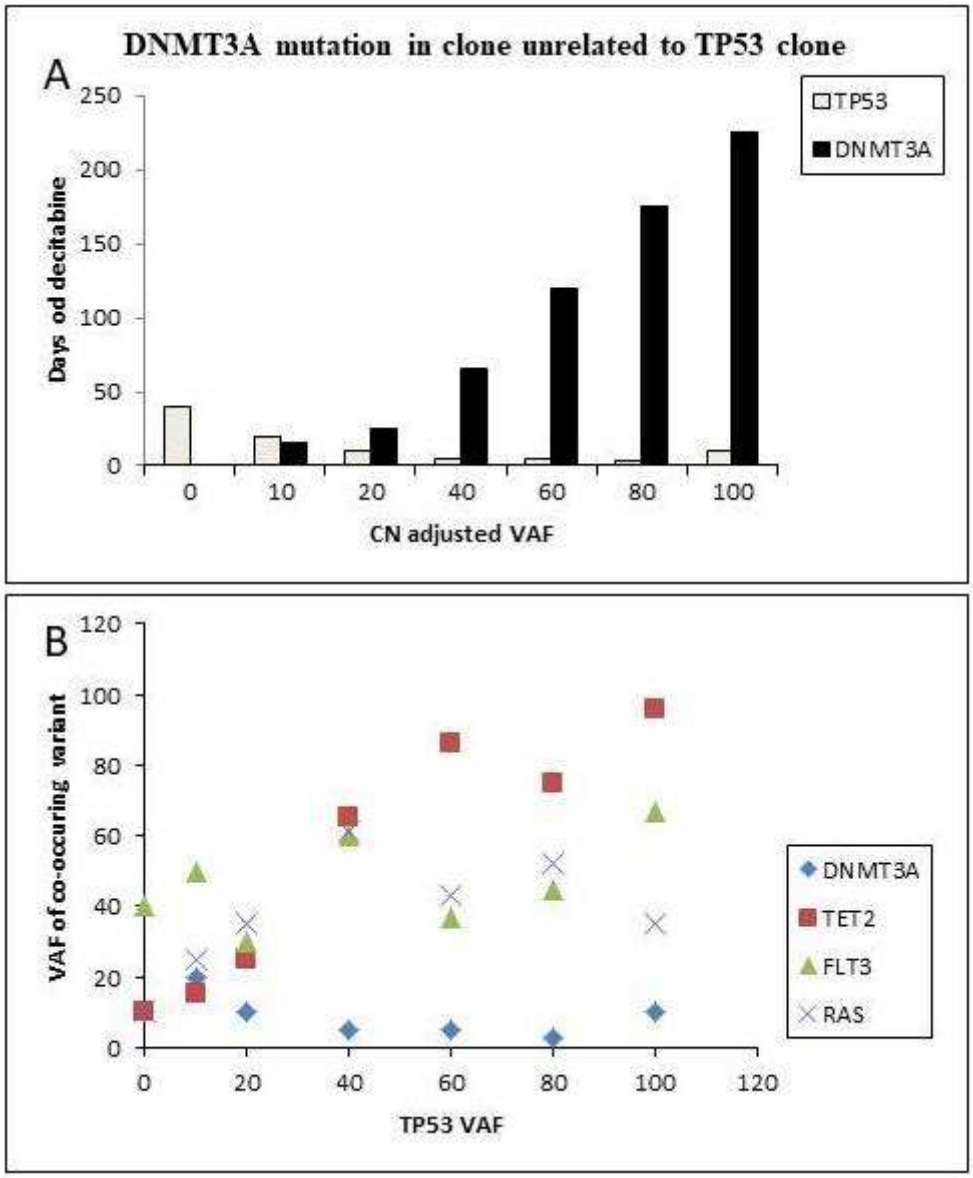

Figure 3. Meta-Analysis of previously reported AML cases having mutation in TP53. (A) Identification of genes involved in AML progression. Columns represents valetudinarian[5]**.**

2. LITERATURE REVIEW

S. Mallick et al. in their study discloses about pancytopenia (weakness, infections, bleeding diathesis) and organ invasion with leukemic cells are indications of acute myeloid leukaemia (AML). Galactorrhea is an unusual symptom of AML. We present a case of AML that manifested as galactorrhea. Excess prolactin secretion can be caused by neurologic problems, neoplastic processes, medicines, hypothyroidism, and chest wall irritation interfering with the hypothalamus pituitary axis. The most prevalent cause of galactorrhea is pituitary prolactinoma, however nonpituitary malignancies such bronchogenic carcinoma, Hodgkin's lymphoma, and T-cell lymphomas have also been linked [6].

S. Jiménez et al. in their study discusses about cancer which is the top cause of death and a serious public health issue. Previous national incidence and death estimates have been confined to tiny samples of the population utilising data from the 1990s or based on a specific year due to China's huge population (1.37 billion). The author’s analysed data from 72 local, population-based cancer registries (2009-2011), representing 6.5 percent of the population, to estimate the number of new cases and cancer deaths for 2015. With high-quality data from an additional number of population-based registries now available through the National Central Cancer Registry of China, the authors analysed data from 72 local, population-based cancer registries (2009-2011), representing 6.5 percent of the population, to estimate the number of new cases and cancer deaths for 2015. Trend studies were conducted using data from 22 registries (2000-2011)[7].

R. L. Siegel et al. in their study discloses about the IDH mutation that results in a neomorphic enzyme, which increases leukemogenesis by causing an aberrant accumulation of R-2-HG. IDH1 R132, IDH2 R140, and IDH2 R172 are the most common IDH mutations found in acute myeloid leukaemia (AML) patients.

The prognostic value of different mutant isoforms varies. IDH inhibitors have had a satisfactory clinical response in AML patients in recent years. As a result, the IDH2 and IDH1 inhibitors developed by Agios Pharmaceuticals, enasidenib and ivosidenib, were authorised by the Food and Drug Administration on August 1, 2017 and July 20, 2018, respectively, for the treatment of adult relapsed or refractory (R/R) AML with IDH2 and IDH1 mutations[8].

3. DISCUSSION

The experiment design is inspired from earlier reported publications [6]. Firstly, ethanolic extracts of rose stems & leaves would be prepared & the AML cell line HL-60 & the normal human embryonic kidney 293 (nHEK-293) cell lines would be treated with various doses of ethanolic extracts for 24 h to determine its IC_{50} value. The results would be analysed for statistical significance. Foetal bovine serum, Cell culture media, MTT, Antibiotics, DMSO etc. that were used for this study were as described earlier. Firstly, the rose leaves & stems (aerial parts) were isolated from *Rosa indica*, & then dried under the sun to remove moisture. The dried rose hips would be pulverized under mortar & pestle to powder form. Ethanolic extracts would be prepared by mixing 30 g of the pulverized powder into 200 ml of ethanol & Soxhlet extraction would be performed to obtain the ethanolic extract.

The ethanolic extract so obtained would be lyophilized & the residues so obtained were kept at 4°C in a method as described earlier. Human AML cell line, HL-60 & normal human embryonic cell lines (nHEK) bought from "National Facility of Animal Tissue & Cell Culture" ("NCCS"), Pune, India. All cells were further subjected to RPMI (Roswell Park Memorial Institute) media to obtain cultures as per manufacturer's instructions. Ethanolic extract of rose was firstly dissolved in DMSO & the solution was then made up with RPMI following which both the HL-60 & nHEK-293 cells (1×10^4) were administered with varying concentrations of Rose's ethanolic extract (0, 10, 20, 30, 40 & 50 µg/ml) for 1 day. Viable cells were confirmed by MTT assay reported earlier. Experiments were performed at least thrice & statistically significance was obtained at $p < 0.05$[9].

3.1 Apoptotic effects of Spergulin A on HL-60 cells:

Growth inhibitory activity of the Ethanolic extract of rose towards AML was observed using the MTT assay. After 24 h treatment, it was found that Ethanolic extract of Rose showed a concentration dependent growth inhibition in HL-60 cell lines, with an IC_{50} value of 44.44 µg/ml (Figure 4) without any growth inhibitory activity on the nHEK-293 cell lines (data not shown). This implies that ethanolic extract of rose unlike on AML cells has no growth inhibitory activity on normal cells[10].

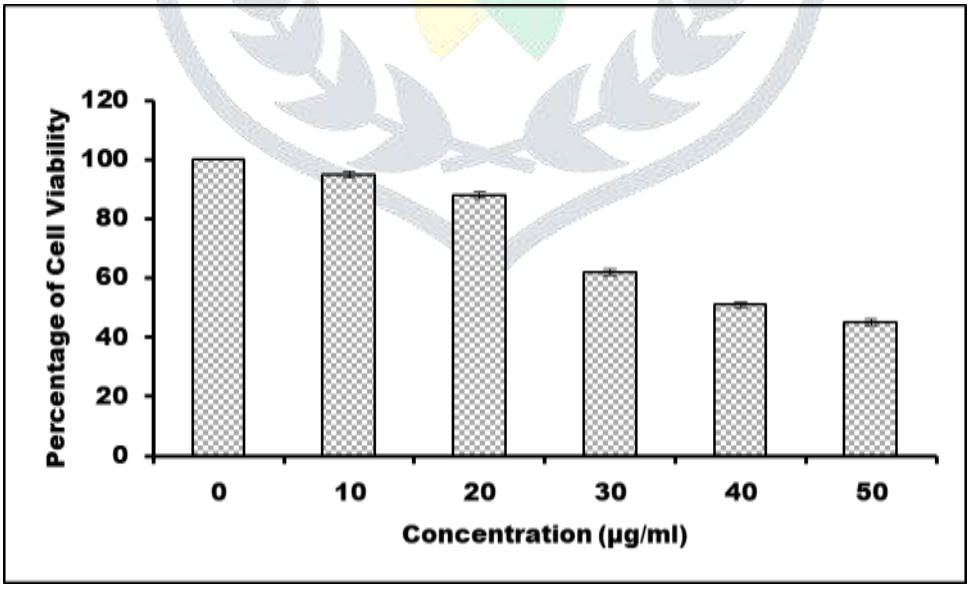

Figure 4: Graph showing growth inhibitory efficacy of the Ethanolic extract of Rose. The AML cells; HL-60 showed amount reliant decrease within percentage of viable cells after 24 h. Values were statistically significant with respect to the control or un-treated cells with P<0.05[11].

5. CONCLUSION

In this paper, the anti-AML activity of an ethanolic extract of Indian Rose has been described. Firstly, Ethanolic extracts of the leaves & stems of the Indian rose (*Rosa indica*) were prepared. The Ethanolic extract was then first dissolved in DMSO & then was reconstituted in RPMI media. The growth inhibitory

activity of the Ethanolic extract of Rose on an AML cell line; HL-60 & on a normal human embryonic kidney cell line; nHEK-293 cells was noted in amount reliant way for 24 h. Ethanolic extract induced growth inhibition on HL-60 cell line in a concentration dependent manner after 24 h, with the IC_{50} value at 44.44 µg/ml whereas no growth inhibition was observed in nHEK cells. These results thereby hint at a possibility of the Ethanolic extract being used as a potential lead agent against AML. As the Indian Rose is commonly available in India, thereby this plant has the potential to produce anti-AML agent at a commercial scale.

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