academicJournals

Vol. 10(42), pp. 783-789, 10 November, 2016
DOI: 10.5897/JMPR2016.6187
Article Number: 47FF22661603
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

In silico physico-chemical evaluation, anti-inflammatory and mcf-7 breast cancer cell line growth inhibition effects of trolline isolated from *Mirabilis jalapa*

Mas'ud Eneji Sadiq[1]*, Hajia Mairo Inuwa[2], Mujeeb-ur-Rehman[3] and Khalid Ahmad[4]

[1]Biochemistry Department, Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria.
[2]Biochemistry Department, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[3]International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan.
[4]Department of Environmental Sciences, COMSATS Institute of Information Technology, Abbottabad, Pakistan.

Received 28 June, 2016; Accepted 30 August, 2016

The *in silico* simulations and predictions approach in evaluation of pharmacokinetic properties of new chemical entities (NCEs) is fast becoming an acceptable trend in natural products research and drug discovery. This paper focuses on the properties of trolline with respect to its anti-inflammatory and MCF-7 breast cancer cell line growth inhibition effects and an attempt to predict physico-chemical drug-like properties *in silico*. The compound was isolated for the first time from aerial parts of *Mirabilis jalapa* and the structure was elucidated by 1D and 2D NMR, FTIR and mass spectroscopic analyses. The compound was screened for anti-inflammatory and anti-MCF-7 cell lines proliferation using chemoluminiscence oxidative burst and MTT assays respectively. Predictions on physico-chemical properties central to oral route drug administration were done using commercial software Admet predictor. Results show anti-inflammatory effects at IC_{50} concentration of 53.8 µg/ml and 48% mortality of breast cancer cell lines at 50 µM concentration. Predictions suggest moderate solubility of 1.72 to 2.92 mg/ml across pH range of 1.6 to 6.8 in simulated solvent systems and effective jejunal permeability of 5.61 x 10^{-4} cm/s. The compound was also predicted to be 60% unbound to plasma proteins and an LD_{50} of 950 mg/kg in rats. The compound, trolline had clinically important bioactive effects, probably possess promising drug-like properties suitable for further high throughput screening.

Key words: ADMET, anti-inflammatory, *in silico* prediction, *Mirabilis jalapa,* NMR, trolline.

INTRODUCTION

The search for new chemical entities (NCEs) has prompted the resurgence in isolation and screening of natural products from diverse sources. Although, the last decade experienced a decline in the interest in natural products research, this has not necessarily resulted in increase in NCEs approved as drugs through combinatorial chemistry aided synthesis (Harvey, 2008). So far, the best-selling therapeutic drugs as at 2012 are

*Corresponding author. E-mail: sadiq.masud@udusok.edu.ng. Tel: +2348026917014.

natural products or derived from them (Newman and Cragg, 2012). Quite a number of medicinal plants and other sources of natural products like the actinomycetes have been identified and are currently researched for their potential as sources of lead candidates for NCEs that are of clinical importance.

The plant, *Mirabilis jalapa* Linn. belongs to the *Nyctagenaceae* family, a perennial herbaceous plant that grows to a height of 50 to 100 cm. The seeds are wrinkled and turn black upon maturity while its beautiful flowers open around 4 O' clock (Nair et al., 2005). The plant commonly called marvel of Peru in English is indigenous to South America and other tropical climates (Taylor, 2005). In Nigeria, it is locally known as *Tonaposo* in Yoruba language (Elufioye et al., 2012) while in Pakistan, it is called *Gul-abbas* (Dhar et al., 1968). Phytochemical investigation reveals the presence of β-sitosterol, ursolic acid, amino acids, β-stigmasterol (Singh and Mittal*,* 2012), phytol (Siddiqui et al., 1994) in the aerial parts while rotenoids (Xu et al., 2010) and trigonelline (Zhou et al., 2012) alkaloid have been characterized from the roots. The plant is known to possess medicinal properties across different cultures. In Brazilian folk medicine for example, the leaf extract is used in treatment of inflammation (Walker et al., 2008). Extracts of the aerial parts are also used as purgative and in the treatment of dysentery (Encarnacion et al., 1998) and diarrhea (Holdsworth, 1992; Comerford, 1996). In Chinese traditional medicine, the root of this plant is used as an anti-diabetic (Zhou et al., 2012). Several literature reports on scientific studies on various parts of the plant suggest the presence of clinically important pharmacologic properties (as claimed), including antibacterial (Eneji et al., 2011), anti-viral (Wong et al., 1992), anti-inflammatory (Nath et al., 2010) and treatment for dysentery (Shaik et al., 2012). Importantly, bioguided isolation of small molecules from *M. jalapa* have also been demonstrated to have clinically important properties including trigonelline (Zhou et al., 2012) and boravinone rotenoids (Xu et al., 2010) isolated from the roots of this plant were shown to have anti-diabetic and in vitro anti-cancer activities, respectively.

The cost of bringing a new drug into the market usually requires heavy budgetary commitments from pharmaceutical industries. Indeed many lead compounds have either been rejected or fail completely at advanced stages of clinical trials. More complicating is the withdrawal of otherwise approved drugs from the market due to evidences emerging from pharmacovigilance (McNaughton et al., 2014; Onakpoya et al., 2015). Developments in *in silico* simulations and predictions using reliable data obtained from laboratory experiments have proven to provide the platform for the advancement of research in screening and testing of NCEs (Yu and Adedoyin, 2003; Moroy et al., 2012) as a viable alternative to circumvent these problems that result in huge investments go down the drain. There are commercially available softwares (Boobis et al., 2002) that predict and simulate the fate of drug components as they interact with different compartments of the body notably, the administration, distribution, metabolism, elimination and toxic (ADMETox) outcomes of NCEs. *In silico* models built around the quantitative structure activity relationship (QSAR) concepts for example, have helped describe physicochemical properties of molecules by reliable laboratory data parameterization and development of molecular descriptors (Butina et al., 2002).

A careful study of reported activities of *M. jalapa* suggest constituents of polar fractions of the various plant parts may contain substances responsible for observed activities (Muthumani et al., 2010; Nath et al., 2010; Oladunmoye, 2012). The isolation of 8,9-dihydroxy-1,5,6,10b-tetrahydropyrrolo[2,1-a]isoquinolin-3(2H)-one (trolline) from polar fractions of aerial parts of *M. jalapa* is reported. This compound was previously isolated from the plants *Salsola collina Pall* (Zhao and Ding, 2004) and *Trollius chinensis* (Li-Jia et al., 2014). The following study seeks to investigate possible anti-inflammatory properties and the ability of this compound to inhibit growth of MCF-7 breast cancer cell lines *in vitro*. the physicochemical drug-like properties of this compound were also evaluated by *in silico* predictions.

MATERIALS AND METHODS

Aerial parts of *M. jalapa* L. plant was collected from the National Veterinary Research Institute (NVRI) Vom, Plateau State Nigeria. A voucher number 2441 was deposited having been identified at the herbarium section of the Biological Science Department, Ahmadu Bello University Zaria, Nigeria. The plant parts were air dried under shade and then crushed using pestle and mortar. The coarse particles size material was then immediately extracted using organic solvent.

Plant extraction and chromatography

Methanol was used to extract 29 g of pulverized plant parts after which hydrated butanol was used to re-extract by partitioning. Fractions F50-F80 obtained from silica column (mesh size 60-120) eluted with 10% MeOH : DCM were pulled together and further separated on flash column, 40% acetone : hexane elution. Fractions F33-F45 from this column were pulled and separated on MCI-gel column eluted with 60% H_2O : MeOH to obtain F23. The fraction F23 was further purified using recycling HPLC (JAI, RP column ODS H-80) at a flow rate of 2.5 ml/min to obtain 12 mg of the pure compound. The sonicated HPLC grade isocratic solvent system used was 60% H_2O : MeOH.

Spectroscopic data

IR (3425, 2925, 1649, 1527, 1452cm^{-1}); ^{1}H NMR (600MHz, MeOD) δ 6.55-6.53 (s, 2H), 4.72 (dd *J*= 18.0, 7.8, 1H, 4.08 (ddd, *J*= 12.9, 6.0, 2.8, 1H), 3.04 (td, *J*= 12.0, 4.3, 1H), 2.76-2.70 (m, 1H), 2.66-2.60 (m, 2H), 2.56 (dd, *J*= 17.9, 7.9, 1H), 2.41-2.36 (m, 1H), 1.80-1.73 (m, 1H); ^{13}C NMR (151MHz, MeOD DEPT) C 175.9, 145.6,

Table 1. NMR spectroscopic data for trolline (600 MHz, MeOD).

Position	δ_C type	δ_H (*J* in Hz)	HMBC[a]
1	28.8 CH_2	1.83-1.73 m 2.66-2.60 m	2, 4, 10b
2	32.7 CH_2	2.56, dd (17.9,7.9)	1, 3, 4
3	175.9 C		
4	58.3 CH	4.72, dd (18.0, 7.8)	1, 6b, 10b
5	38.6 CH_2	4.08, ddd (12.9, 6.0, 2.8) 3.04, td (12.0, 4.3)	3, 4, 6, 6b
6	28.8 CH_2	2.76-2.70 m 2.66-2.60 m	5, 6b, 10b
6b	125.5 C		
7	116.2 CH	6.55-6.53 s	6, 9, 10b
8	145.5 C		
9	145.6 C		
10	112.4 CH	6.55-6.53 s	4, 6b, 8
10b	129.8 C		

[a]Proton-carbon HMBC for 2-3 bond connectivity.

145.5, 129.8, 125.5; CH 116.2, 112.4, 58.3; CH_2 38.6, 32.7, 28.8, 28.8; HREIMS M* 219.0882 Molecular formula $C_{12}H_{13}NO_3$; Ring plus double bond (RDB) analysis =7.

In vitro biological screening

Anti-inflammatory assay

Chemoluminiscence method as described by Helfand et al. (1982) was adopted. Briefly, 25 µL from each of the prepared trolline concentrations of 1, 10 and 100 mg/ml was incubated with 25 µL of diluted whole blood $HBSS^{++}$ (Hanks Balanced Salt Solution, containing calcium chloride and magnesium chloride) [Sigma, St. Louis, USA]. Incubation of prepared cells and control (containing no test compound) was done at 37°C for 15 min in white half area 96 well plates (Costar, NY, USA) in a luminometer thermostat chamber (Labsystems Helsinki, Finland). Exactly 25 µL each of opsonized zymosan (Fluker Buchs, Switzerland) and luminol (Research Organics Cleveland, USA) were added into each well except for blank wells containing only $HBSS^{++}$. Readings indicating reactive oxygen species (ROS) levels were recorded in luminometer and expressed as relative light units (RLU). Ibuprofen was used as standard control anti-inflammatory drug.

MCF-7 breast cancer cell lines MTT assay

The method as described by Scudiere et al. (1988) involves seeding 96 well tissue culture treated flat bottom plates with precultured MCF-7 cell lines in Dulbecco's modified Eagle medium (5% CO_2 incubator at 37°C). After incubation for 24 h, 50 µM of trolline was added to each well in triplicate and incubated for 48 h. The tetrazolium salt MTT (3-(4,5-dimethylthiazol-yl)-2,5-diphenyl tetrazolium bromide) was added (200 µL) after washing the cells and then incubated for 3 h at 37°C. Formazan crystals formed were dissolved in 100 µL DMSO and absorbance taken at 570 nm using micro-plate reader (Spectra Max plus, Molecular devices, CA, USA). Results were recorded as percent growth inhibition of viable cells. Prepared alongside was doxorubicin as the control drug.

In silico evaluation

The physicochemical properties of trolline were evaluated using Admet predictor software, a commercially available licensed property of Simulations plus Inc Lancaster, USA. SMILE notations of isolated compound and controls used in the assays described was used to create the input files. Predictions were carried out on the 2D generated structures of the compounds using default settings of pH 7.4.

RESULTS

NMR assignments

As presented in the spectroscopic data, the molecular formula as determined from HREI-MS is $C_{12}H_{13}NO_3$ with pseudo molecular ion peak appearing at 218 m/z. The molecular mass was confirmed by ESI-MS with $[M+H]^+$ 220 m/z. The FTIR analysis is in conformity with the odd numbered molecular mass suggesting the presence of N atom occurring as a tertiary amide functional group. NMR ^{13}C (DEPT) analysis identifies presence of 12 carbons defined thus, 5 quartenary (1 carbonyl and 4 aromatic carbons), 3 methine (2 quartenary and 1 aliphatic) and 4 aliphatic methylene carbons. Presented in Table 1 is the NMR assignment using 2D spectroscopic data. The aromatic methine protons are assigned to C7 (δ 116.2) and C10 (δ 112.4) in *para* positions due to the tall singlet observed in ^1H NMR. The HMBC experiment (Table 1) described in the schematic (Figure 1) shows the 2-3 bond connectivity.

Bioactivities

Figure 2 presents the bioactive effects of trolline on anti-

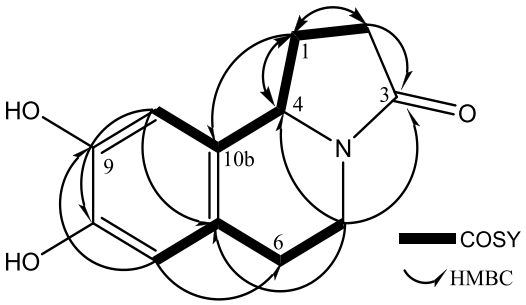

Figure 1. HMBC correlations (^{1}H - ^{13}C) for the compound, trolline.

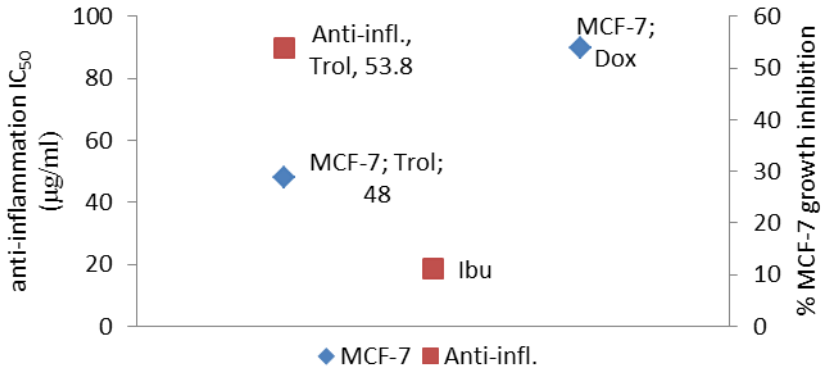

Figure 2. Anti-inflammation and anti-MCF-7 proliferative effects of trolline.

inflammation and growth inhibition of MCF-7 breast cancer cell lines. The results show IC_{50} of 53.8 µg/ml concentration on anti-inflammatory effects and 48% inhibition of MCF-7 cell lines proliferation at 50 µM.

In silico predictions

Predictions on solubility in different solvent systems as presented in Figure 3 indicate that trolline is moderately soluble in gastric and intestinal fluids. Prediction models suggest the compound to be soluble in aqueous environment over a pH range of 1.6 to 6.8. Models for permeability predictions (Figure 4) indicate 5.61 $x10^{-4}$ cm/s effective jejunal permeability for trolline which is relatively high as compared to the cytotoxic drug doxorubicin predicted to have <0.5 x 10^{-4} cm/s, suggesting a low permeability property for this compound.

Plasma protein binding properties of trolline presented in Table 2 indicate the compound exist more in the free state rather than bound to plasma proteins. Trolline preferentially sequester within the blood rather than plasma (RBP = 1.25) and has minimal tissue distribution (as the model predicts).

DISCUSSION

The concentration of 53.8 µg/ml (0.25 mM) recorded for anti-inflammation could be considered significant as it falls within minimum concentration (IC_{50}) of 156 to 400 µg/ml (Choudhary et al., 2009). Inflammation is a normal physiologic response to disease and tissue damage. There are emerging evidences however, linking chronic inflammation and carcinogenicity (Coussens and Werb, 2002; Coussens et al., 2013), notably the chemokine system and recruitment of tumor related macrophages (Mantovani et al., 2010). Although, 90% mortality was recorded for doxorubicin at 50 µM (Figure 2), non-target cells cytotoxicity and other contra-indications associated with use of doxorubicin are limitations to the use of this compound (Alexieva et al., 2014). Comparatively however, and at similar concentrations, trolline was observed to inhibit 48% growth of MCF-7 breast cancer cells. Compounds with pyrrolo[2,1-a]isoquinoline structure are reported to have hypotensive and anti-tumor activities (Mikhailovskii and Shklyaev, 1997) as well as antibacterial properties (Wang et al., 2004). Oleracein E (the antipode of trolline) has been reported to exhibit potent DPPH radical scavenging activity (Yang et al., 2009). Other cytotoxic compounds characterized from *M.*

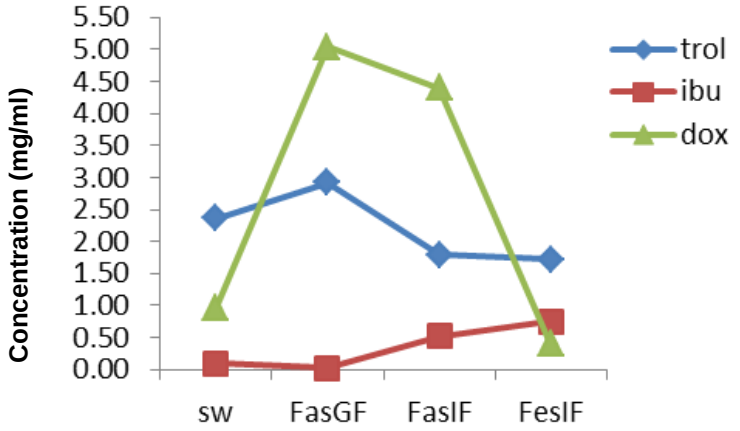

Figure 3. Prediction models on compound solubility. Sw = native solubility in water; FasGF = solubility in simulated fasted state gastric fluid; FasIF = solubility in simulated fasted state intestinal fluid; FeSIF = solubility in simulated fed state intestinal fluid; Simulations Plus.

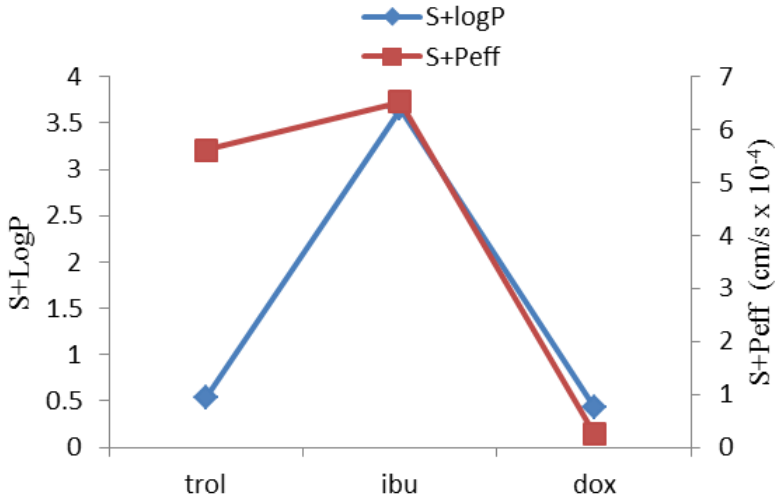

Figure 4. Prediction models on jejunal permeability. S+logP = octanol-water partition coefficient; s+Peff = human jejunal effective permeability.

Table 2. Predictions on protein binding, volume of distribution and blood-plasma concentrations.

Compound	PrUnbnd (%)	VD (L/Kg)	RPB
Trolline	60.23	0.95	1.25
Ibuprofen	2.10	0.34	0.58
Doxorubicin	21.56	11.48	0.99

PrUnbnd: % unbound proteins; VD: volume of distribution; RPB: blood-plasma ratio.

jalapa, notably the cytotoxic rotenoids mirabijalones and boeravinones have been shown to have growth inhibition effects against several cell lines (Wang et al., 2002; Xu et al., 2010).

Predictions on absorption properties for trolline conforms with laboratory findings using human Caco-2 cell monolayer model (Li-Jia et al., 2014), suggesting trolline to be moderately absorbed in the GIT. Similar trends in prediction results were observed for ibuprofen used as control for anti-inflammation properties; shown to be highly lipophilic and permeable to the jejunum (Figure 4) but relatively low in solubility (Figure 3). Ibuprofen traversing the GIT is a pH dependent phenomenon, permeable through walls of the stomach but not readily soluble; thus, limiting its entry into systemic circulation (Patel et al., 2011).

Table 3. Predictions on compound LD_{50} and TD_{50} in rats.

Compound	LD_{50} (mg/Kg)	TD_{50} (mg/Kg/day)
Trolline	950.00	107.23
Ibuprofen	1175.37	82.27
Doxorubicin	463.14	19.17

LD_{50} = LD50 for lethal rat acute toxicity; TD50 = TD50, defined as the oral dose of a compound required to induce tumors in 50% of a rat population after exposure over a standard lifetime.

Key parameters for drugs intended for oral administration are solubility and permeability. Dissolution of target drug dose around site of absorption increases the propensity for bioavailability (Amidon et al., 1995). Thus, early identification of low solubility drug candidates estimated at 40% of new drugs (Dahan and Muller, 2012) is important in the long term downstream screening. High jejunal permeability was predicted for trolline and was also shown to be present in blood more in the free state rather than bound to plasma proteins. Model prediction on the control drug doxorubicin (Table 2) however indicates high volume of tissue distribution, which is consistent with literature reports (809-1214 Lm^{-2}) on the pharmacokinetics of doxorubicin with a distribution half-life of 5 min (National Center for Biotechnology Information, 2016).

Existence of drugs in free unbound state allows for effective interactions with target receptors thus increasing drug efficacy. Alterations in plasma protein binding inevitably affect dose formulations, clearance time and systemic distribution of drugs (Scheife, 1989; Roberts et al., 2013). The quantitative structure activity relationships (QSAR) methods used in developing descriptors for in-silico predictions provide insights into the probable behaviour of NCEs with plasma proteins (Saiakhov et al., 2000; Ghafourian and Amin, 2013).

Communities, especially in developing countries where use of medicinal plant is a common cultural practice, these plants are often not regarded as food but are however consumed at concentrations exceeding what is obtainable in clinical practice. Predictions (Table 3) on lethal rat acute toxicity (LD_{50}) observed for trolline was 950 mg/kg; and a life time oral dose administration (TD_{50}) of 107.23 mg/kg/day is likely to induce tumors (in rats). Considering standard practice in drug dosage formulations, we assume from predicted values as well as moderate toxicity against MCF-7 breast cancer cells (*in vitro*), trolline may not constitute a threat to normal body homeostasis but construed to play functional roles in the long term prevention of early onset of disease and other forms of tissue damage. However, this is a subject matter for clinical verification. *In silico* predictions on administration, distribution, metabolism and elimination (ADME) of drugs in modern day research has the advantage of limiting time and reducing the cost of searching for NCEs with drug-like potentials (Butina et al., 2002; Kapetanovic, 2008).

Conclusion

In silico simulation and prediction are becoming more efficient and fast gaining popularity and robust applicability in pharmaceutical research. The development of proficient machine language programming has revolutionalized this tool as a necessary cost effective step in drug discovery. The compound, trolline was demonstrated to have clinically important bioactive properties and predictions on the physico-chemical properties of this compound are quite promising in terms of its characteristic drug like properties.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors acknowledge the support of TWAS-ICCBS in providing fund and work place for this research. They are also grateful to Simulations-plus inc. Lancaster, USA for providing access to Admet predictor software.

REFERENCES

Alexieva B, Sainova I, Pavlova V, Markova T, Valkova I, Nikolova E (2014). Insights into Mechanisms of Doxorubicin Cardiotoxicity. J. Phys. Pharm. Adv. 4(3):342-348.

Amidon GL, Lennernas H, Shah VP, Crison JR (1995). A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12:413-420.

Boobis A, Gundert-Remy U, Kremers P, Macheras P, Pelkonen O (2002). In silico prediction of ADME and pharmacokinetics: Report of an expert meeting organised by COST B15. Eur. J. Pharm. Sci. 17(4):183-193.

Butina D, Segall MD, Frankcombe K (2002). Predicting ADME properties in silico: methods and models. Drug Discov. Today 7(11):S83-S88.

Choudhary MI, Jalil S, Nawaz SA, Khan KM, Tareen RB (2009). Anti-inflammatory and lipooxygenase inhibitory compounds from vitex agnus-castus. Phytother Res. 23(9):1336-1339

Comerford SC (1996). Medicinal plants of two Mayan healers from San Andŕes, Peten, Guatemala. Econ. Bot. 50:327-336.

Coussens LM, Werb Z (2002). Inflammation and cancer. Nature 420(6917):860-867.

Coussens LM, Zitvogel L, Palucka AK (2013). Neutralizing tumor-promoting chronic inflammation: a magic bullet? Science 339(6117):286-291.

Dahan A, Miller JM (2012). The solubility–permeability interplay and its implications in formulation design and development for poorly soluble drugs. AAPS J. 14(2):244-251.

Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Ray C (1968). Screening of Indian plants for biological activity: I. Indian J Exp. Biol. 6(4):232-247.

Elufioye TO, Oladele AT, Cyril-Olutayo CM, Agbedahunsi JM,

Adesanya SA (2012). Ethnomedicinal study and screening of plants used for memory enhancement and antiaging in Sagamu, Nigeria. Eur. J Med Plants 2(3):262.
Encarnacion DR, Virgen M, Ochoa N (1998). Antimicrobial activity of medicinal plants from Baja California Sur (Mexico). Pharm. Biol. 36:33-43.
Eneji SM, Inuwa HM, Ibrahim S, Ibrahim AB Abdulfattah A (2011). In vitro assessment of bioactive components of *Mirabilis jalapa* ethanolic extract on clinical isolates of *Salmonella typhi* and *Bacillus cereus*. Afr. J. Biotechnol. 10(71):16006-16011.
Ghafourian T, Amin Z (2013). QSAR models for the prediction of plasma protein binding. BioImpacts 3(1):21-27.
Harvey AL (2008). Natural products in drug discovery. Drug Discov. Today 13(19):894-901.
Holdsworth DK (1992). A preliminary study of medicinal plants of Easter Island, South Pacific. Int. J. Pharmacogn. 30:27-32.
Kapetanovic IM (2008). Computer-aided drug discovery and development (CADDD): in silico-chemico-biological approach. Chem. Biol. Interact. 171(2):165-176.
Li-Jia L, Xiu-Wen W, Ru-Feng W, Yu-Shuai P, Xin Y, Jun-Xiu L (2014). Absorption properties and mechanism of trolline and veratric acid and their implication to an evaluation of the effective components of the flowers of Trollius chinensis. Chin. J. Nat. Med. 12(9):700-704.
Mantovani A, Savino B, Locati M, Zammataro L, Allavena P, Bonecchi R (2010). The chemokine system in cancer biology and therapy. Cytokine Growth Factor Rev. 21(1):27-39.
McNaughton R, Huet G, Shakir S (2014). An investigation into drug products withdrawn from the EU market between 2002 and 2011 for safety reasons and the evidence used to support the decision-making. BMJ Open 4(1):e004221.
Mikhailovskii A G, Shklyaev VS (1997). Pyrrolo [2, 1-a] isoquinolines (review). Chem. Heterocycl. Compd. 33(3):243-265.
Moroy G, Martiny VY, Vayer P, Villoutreix BO, Miteva MA (2012). Toward in silico structure-based ADMET prediction in drug discovery. Drug Discov. Today 17(1):44-55.
Muthumani M, Devi P, Meera R, Kameswari B, Eswarapriya B (2010). In vitro antimicrobial activity of various extracts of Mirabilis jalapa leaves. Int. J. Microbiol. 7(2):120-124.
Nair R, Kalariyat T, Chanda S (2005). Antibacterial activity of some selected Indian medicinal flora. Turk. J. Biol. 29:41-47.
Nath LR, Manjunath KP, Savadi RV Akki KS (2010). Anti-inflammatory activity of *Mirabilis jalapa* linn. Leaves. J. Basic Clin. Pharm. 1(2):93-96.
National Center for Biotechnology Information (NCBI) (2016). PubChem Compound Database; CID=31703, Available at: https://pubchem.ncbi.nlm.nih.gov/compound/31703#section=Top Accessed Jan. 18, 2016.
Newman DJ, Cragg GM (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. J. Nat. Prod. 75(3):311-335.
Oladunmoye M (2012). Antioxidant, free radical scavenging capacity and antimicrobial activities of Mirabilis jalapa. J. Med. Plants Res. 6(15):2909-2913.
Onakpoya IJ, Heneghan CJ, Aronson JK (2015). Delays in the post-marketing withdrawal of drugs to which deaths have been attributed: a systematic investigation and analysis. BMC Med. 13(1):26.
Patel SK, Kumar D, Waghmode AP, Dhabale AS (2011). Solubility enhancement of ibuprofen using hydrotropic agents. Int. J. Pharm. Life Sci. 2(2):542-545.
Roberts JA, Pea F, Lipman J (2013). The clinical relevance of plasma protein binding changes. Clin. Pharmacokinet. 52(1):1-8.
Saiakhov RD, Stefan LR, Klopman G (2000). Multiple computer-automated structure evaluation model of the plasma protein binding affinity of diverse drugs. Perspect. Drug Discov. Des.19(1):133-155.
Scheife RT (1989). Protein binding: what does it mean? Ann. Pharmacother. 23(7-8)-S27-S31.
Shaik S, Rajendra Y, Jaya Chandra Reddy P (2012). Phytochemical and pharmaclogical studies of mirabilis jalapa linn. Int. J. Pharm Technol. 4(2):2075-2084.
Siddiqui BS, Adil Q, Begum S, Siddiqui S (1994) Terpenoids and steroids of the aerial parts of *Mirabilis jalapa* Linn. Pak. J. Sci. Ind. Res. 37:108-110.
Singh M, Mittal SK (2012). Mirabilis Jalapa-A Review. Int. J. Pharmaceut. Med. Appl. Sci. 1(3):22-43.
Taylor L (2005). The healing power of rainforest herbs. Tropical Plant Database. http://www.rain-tree.com. Accessed 11th April, 2016.
Walker CIB, Trevisan G, Rossato MF, Franciscato C, Pereira ME, Ferreira J, Manfron MP (2008). Antinociceptive activity of *Mirabilis jalapa* in mice. J. Ethnopharmacol. 120(2):169-175.
Wang RF, Yang XW, Ma CM, Cai SQ, Li JN, Shoyama Y (2004). A bioactive alkaloid from the flowers of *Trollius chinensis.* Heterocycles 63(6):1443-1448.
Wang YF, Chen JJ, Yang Y, Zheng YT, Tang SZ, Luo SD (2002). New rotenoids from roots of *Mirabilis jalapa.* Helv. Chim. Acta 85(8):2342-2348.
Wong RN, Ng TB, Chan SH, Dong TX, Yeung HW (1992). Characterization of Mirabilis antiviral protein--a ribosome inactivating protein from *Mirabilis jalapa* L. Biochem. Int. 28(4)585-593.
Xu JJ, Chen Qing, Lv YP, Liu YM, Liu Y, Ye-Gao Chen (2010). Cytotoxic rotenoids from *Mirabilis jalapa.* Chem. Nat. Compd. 46(5):792.
Yang Z, Liu C, Xiang L, Zheng Y (2009). Phenolic alkaloids as a new class of antioxidants in *Portulaca oleracea*. Phytother. Res. 23(7):1032-1035.
Yu H, Adedoyin A (2003). ADME–Tox in drug discovery: integration of experimental and computational technologies. Drug Discov. Today 8(18):852-861.
Zhao YX, Ding XB (2004). Studies on the alkaloids from *Salsola collina* Pall. Acta Pharm. Sin. B. 39(8):598-600.
Zhou J, Chan L, Zhou S (2012). Trigonelline: A plant alkaloid with therapeutic potential for diabetes and central nervous system disease. Curr. Med. Chem. 19(21):3523-3531.